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## *Progress Report ...*

# **Eastern Utilization Research and Development Division**

## **Marketing and Nutrition Research**

**July 1, 1970**

**Agricultural Research Service  
U. S. DEPARTMENT OF AGRICULTURE**



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Issued December 1970

PROGRESS REPORT  
OF THE  
EASTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

INTRODUCTION

"Research was a foundation stone of the Department in 1862. Through every type of crisis from that date onward it had helped meet the changing needs of agriculture and had aided the American farmer to become the most productive of any in the world" (Century of Service" Yearbook of Agriculture 1963, p. 367).

The ten current goals of agricultural research, as stated in "A National Program of Research for Agriculture", a report of a study sponsored jointly by the Association of State Universities and Land Grant Colleges and the U.S. Department of Agriculture, are supported by a wide range of activities which directly or indirectly contribute to better living for everyone. The research effort of the Eastern Utilization Research and Development Division is applied to particular problem areas of goals IV, VI, VII, IX and X as listed:

- I. Insure a stable and productive agriculture for the future through wise management of natural resources.
- II. Protect forests, crops and livestock from insects, diseases, and other hazards.
- III. Produce an adequate supply of farm and forest products at decreasing real production costs.
- VI. Expand the demand for farm and forest products by developing new and improved products and processes and enhancing product quality.
  1. New and improved meat, milk, eggs and other animal food products.
  2. New and improved non-food animal products.
  3. New and improved fruit and vegetable products and byproducts.
  4. New and improved feed, textile, and industrial products from field crops.
  5. New and improved forest products.
- V. Improved efficiency in the marketing system.
- VI. Expand export markets and assist developing nations.
  1. Foreign market development.

- VII. Protect consumer health and improve nutrition and well-being of the American people.
  - 1. Protect food and feed supplies from harmful microorganisms and naturally occurring toxins.
  - 2. Reduction of hazards to health and safety.
  - 3. Human nutrition.
- VIII. Assist rural Americans to improve their level of living.
- IX. Promote community improvement including development of beauty, recreation, environment, economic opportunity, and public services.
  - 1. Alleviation of soil, water and air pollution and disposal of wastes.
  - 2. Improved income opportunities in rural communities.
- X. Enhance the national capacity to develop and disseminate new knowledge and new or improved methodology for solving current problems or new problems that will arise in the future.
  - 1. Under this goal, supporting research is conducted in the problem areas to gain new fundamental knowledge, providing the building blocks to solve problems facing agriculture now and in the future.

The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup and Eastern fruits and vegetables including potatoes. This report is organized according to these commodities and to the research problem areas pertinent to each commodity.

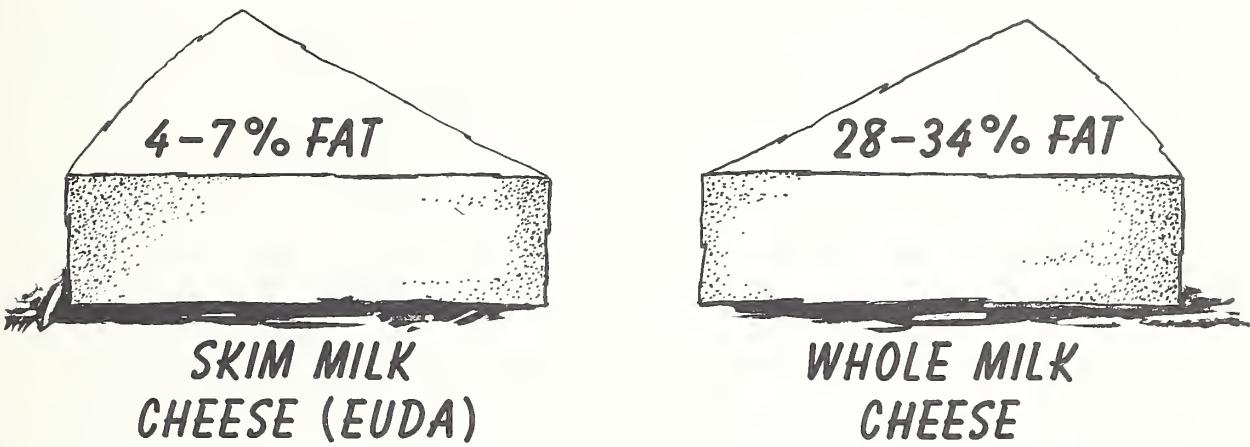
In their research, Division scientists cooperate with representatives of colleges and universities, state experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. In addition, the Division supervises domestic research contracts and grants, and the program is supplemented by a variety of research projects in foreign countries under Public Law 480 grants. Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Following are some examples of recent developments based on research at the Eastern Division and on Division-supervised research that typify such achievements.

## SELECTED SAMPLES

### NEW LOW-FAT CHEESE DEVELOPED

A new skim milk semisoft cheese with low fat content has been developed by Department scientists. The cheese has been made on a pilot plant scale at the Department's Beltsville laboratories and has been test marketed. Consumers liked the new cheese, called EUDA cheese because it was developed at the Eastern Utilization Division of the Department. Their purchases of EUDA in a twelve week period amounted to about eight percent of all other cheese varieties in the survey, while not significantly affecting the sales of other varieties. This development has stimulated considerable public and commercial interest in low-fat cheese with over 100 cheese manufacturers and food processors having received manufacturing instructions for making it. A well known cheese company has requested licensing under a government patent issued to cover the production of EUDA cheese and has indicated its intent to manufacture and sell this product in interstate commerce. Several other commercial companies are now producing their own versions of a low-fat cheese, relying heavily on research results from EU for their development.

## EUDA SKIM MILK CHEESE



- FLAVORFUL WITH LOW FAT
- IN MARKET TEST.....
  - SHOWED GOOD CONSUMER ACCEPTANCE
  - 75% OF BUYERS PLANNED ADDITIONAL PURCHASES
  - SALES WERE 8% OF ALL CHEESE PURCHASES WITHOUT AFFECTING SALES OF OTHER CHEESES

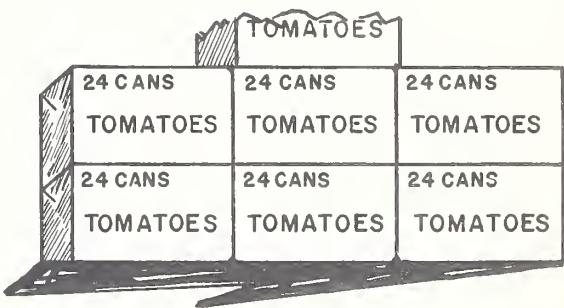
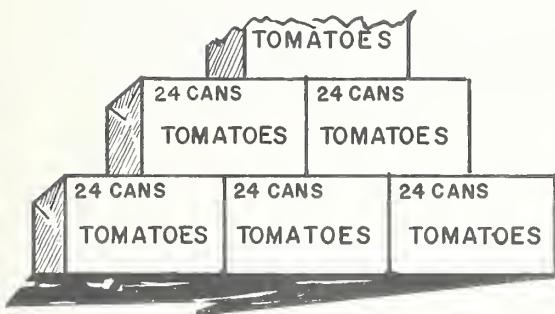
BETTER PRODUCT AND LESS WASTE IN TOMATO PROCESSING

Department scientists and engineers have found that removing natural wax from the skins of fruits and vegetables before lye peeling contributes to better quality, improved yield, lower cost and less waste. The new process permits successful application of lye peeling to certain soft vegetables and to many fruits that previously could not be peeled effectively in this way. A hot organic solvent is used in the new process to remove the wax layer that causes resistance to lye treatment, and formerly required so much heat treatment for lye penetration that damage to quality or excessive loss of product resulted.

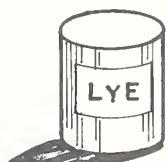
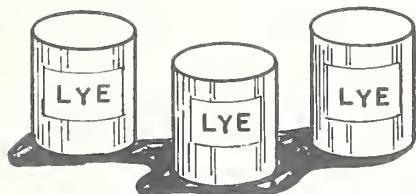
The process has already been adopted by a tomato packer for lye-resistant mechanically harvested tomatoes. In the first season of use the yield of premium-pack tomatoes was increased by 17.5%, waste disposal was reduced by 40%, lye costs were lowered by 67%, and the overall quality of the product was improved. It is estimated that the use of this process returned \$870,000 to the packer after deducting equipment costs and benefited the consumer by providing higher quality products. Other canning companies will use the process in the 1970 season.

# DEWAXING SPEEDS LYE PEELING COMMERCIAL USE IN TOMATO PACKING SHOWS:

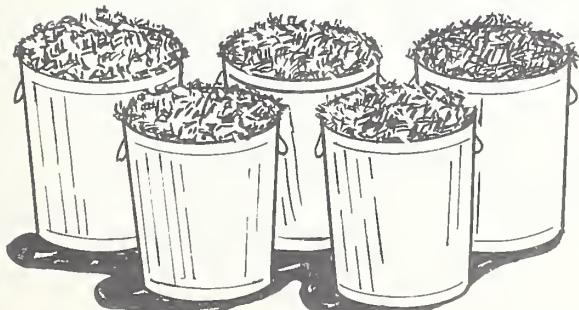
MORE CASES OF A BETTER PRODUCT  
PER TON OF TOMATOES



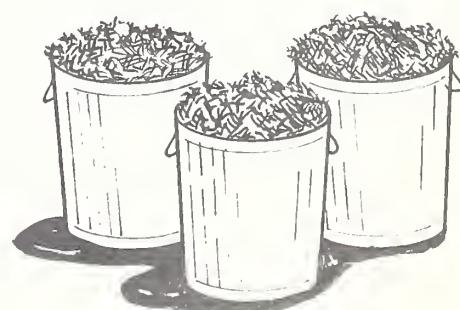
REDUCED LYE USE



LESS WASTE FOR DISPOSAL



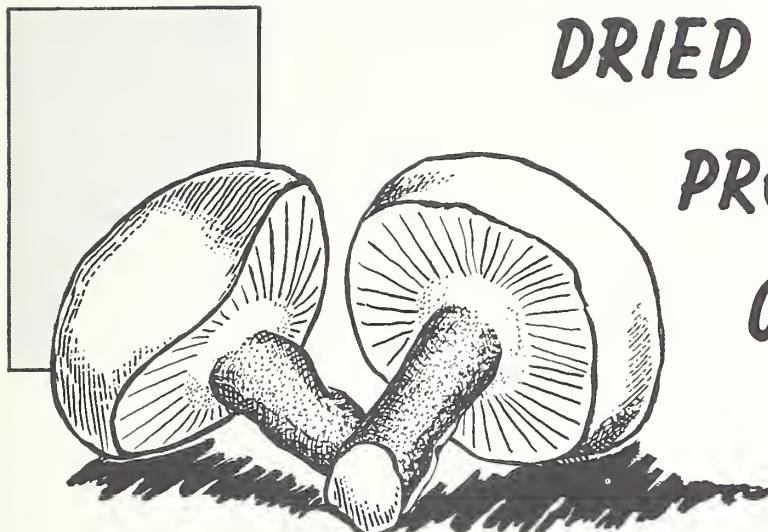
OLD PROCESS



NEW PROCESS

AIR-DRIED MUSHROOMS

A new process for hot-air drying of cultivated mushrooms was developed by Department scientists in order to increase consumption of domestic mushrooms. This will help to relieve the economic pressures imposed on American mushroom growers by the increasing influx of imported processed mushrooms. The process yields a product similar in flavor to the very expensive freeze-dried mushrooms at about one-half the cost. The product is stable in sealed containers. Blanching, which was necessary in the previous process, is not required to inhibit enzyme action and the dried mushrooms have an attractive light color. A large mushroom canner now dries cultivated mushrooms by this process and supplies them for use in commercial food operations.



## DRIED MUSHROOM PROCESS GOES COMMERCIAL

- FLAVOR AND COLOR SIMILAR TO FREEZE-DRIED PRODUCT AT HALF THE COST.
- STABLE PRODUCT.
- HELPS DOMESTIC PRODUCERS MEET COMPETITION FROM IMPORTED PRODUCTS.

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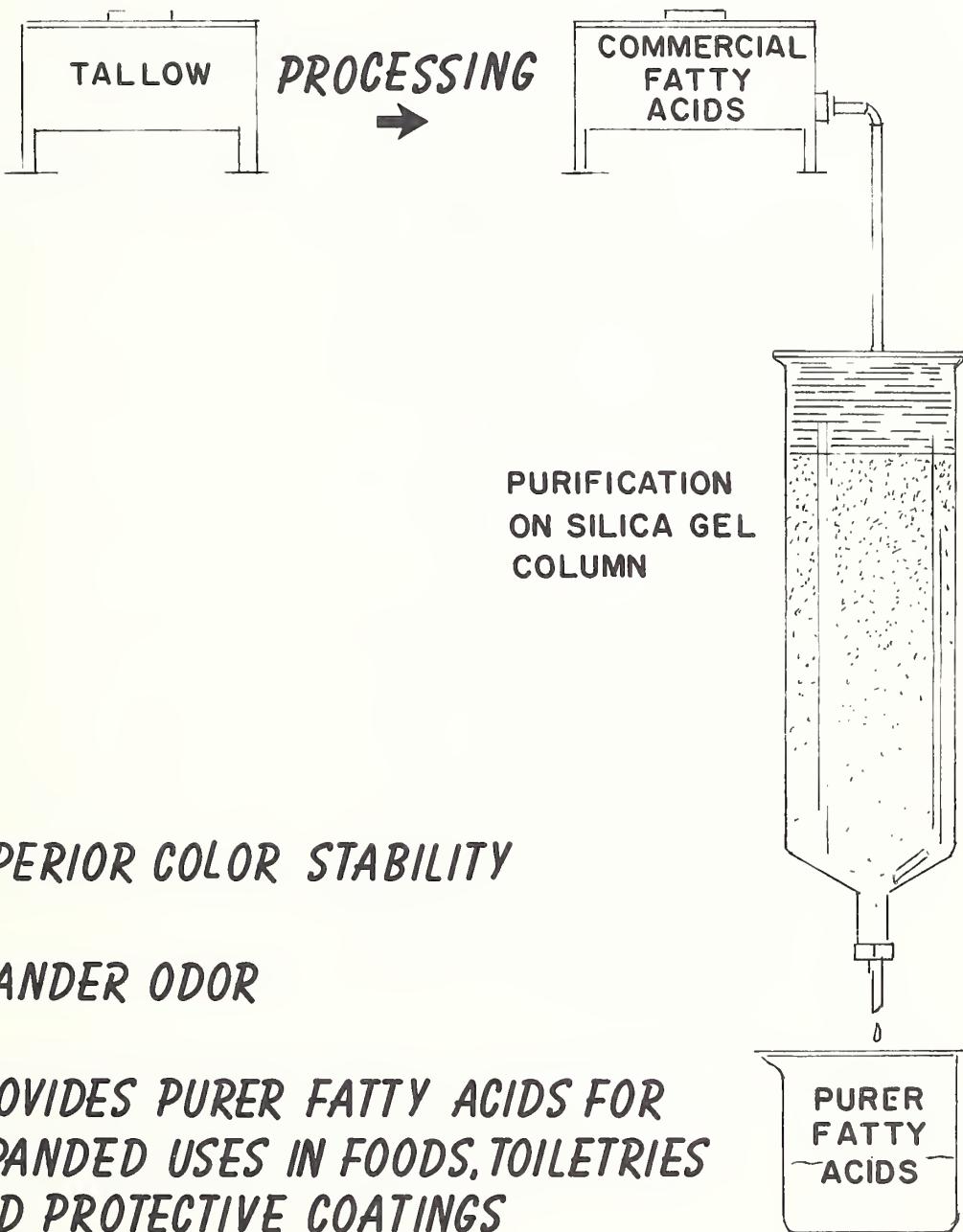
MUSHROOM GROWER AND PACKER IS USING  
PROCESS.

PRODUCT USED IN COMMERCIAL FOOD  
OPERATIONS.

A NEW METHOD FOR THE IMPROVEMENT OF COLOR AND ODOR STABILITY OF  
COMMERCIAL FATTY ACIDS

Commercially available fatty acids, particularly those made from natural products, such as beef tallow and soybean oil soap stock, have poor color and odor stability as shown by a tendency to develop an objectionable odor and dark color during heating or reaction with other chemicals. For this reason, their use is limited in such products as foods, toiletries and protective coatings. A new procedure has been devised under a Grant project supported by the Department for obtaining industrial fatty acids that have greater color and odor stability than the current products. In the new procedure, a solution of the crude fatty acid mixture obtained from processing of natural products is passed through a column containing silica gel, a solid substance that selectively absorbs impurities. The fatty acids eluted from the column have a superior color stability, less odor and do not require further purification. Furthermore, the column material can be regenerated and re-used. The current U.S. production of commercial fatty acids is approximately 500 million pounds per year and can be expected to increase greatly if this method is adopted widely by industry. One company plans to utilize this process for upgrading selected fatty acid products now being manufactured. This company has also indicated an intent to obtain a license since application for a public service patent has been made by the Department.

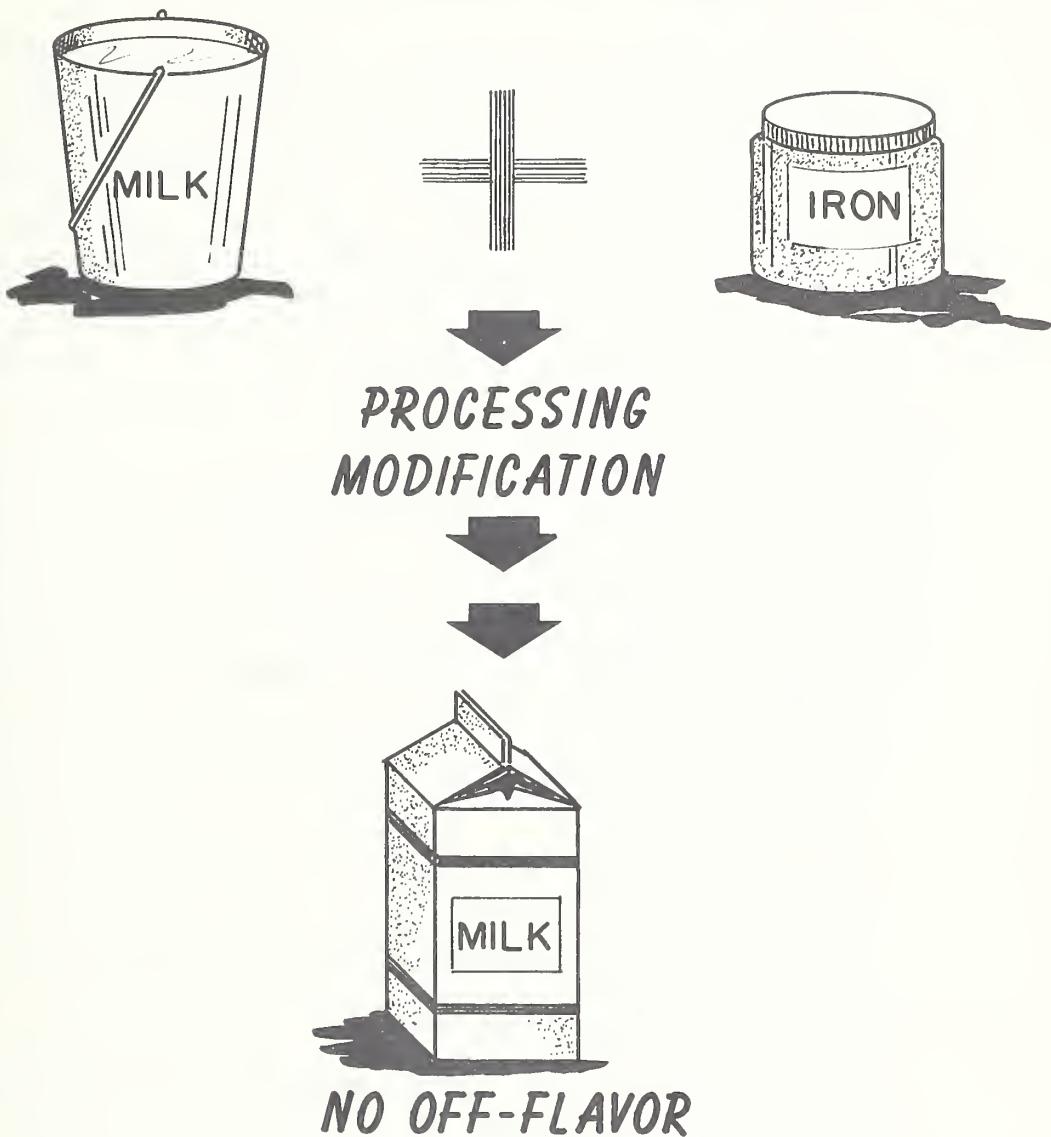
# PREPARATION OF PURER FATTY ACIDS



ENRICHMENT OF WHOLE MILK WITH IRON

Iron and calcium are nutrients most often found to be below recommended daily allowances in the diets of several population groups. In a large number of children under 3 years of age, daily iron intake is estimated to be 50 percent below recommended amounts. Enrichment of milk with iron could be a major means of correcting iron deficiency in the diet. ARS scientists have now established and can recommend to dairies suitable procedures for incorporating 10 milligrams of iron per quart of whole milk. This level is appropriate for improvement of nutritional quality when used by deficient population groups. Changes in processing are necessary to avoid off-flavors when iron is added to milk but these changes can be instituted easily by dairies. More widespread acceptance and use of iron-fortified milk should result in improving the nutrition and hence the health and well-being of deficient individuals and groups.

# IRON ENRICHMENT OF MILK



- PROCESSING CHANGES ALLOW IRON ENRICHMENT WITH ACCEPTABLE FLAVOR
- NO SPECIAL EQUIPMENT OR HANDLING REQUIRED FOR USE BY DAIRIES

## DAIRY PRODUCTS UTILIZATION

### Problems and Objectives

Although consumption of low fat milk has increased, the per capita milk equivalent of all fluid items has shown a downward trend for more than 10 years. These decreases have adverse implications to nutrition of the general population and to economic stability of the dairy industry. Butter remains in excess and cheese whey is a serious stream pollution problem. Research on utilization of milk emphasizes the development of better processing procedures; improved dairy products, including more stable and easily transported products; new and increased food uses, especially for those milk components in excess supply; and economical disposal of dairy wastes.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Develop milk products of enhanced nutritional properties.
2. Develop dry whole and concentrated milks of beverage quality.
3. Prevent the formation of or eliminate objectionable flavors in dairy products, and stabilize and intensify desirable flavors.
4. Prevent or eliminate contamination of dairy products with disease-producing microorganisms.
5. Develop food uses and more economical disposal processes for cheese whey.
6. Develop new cheese and milk fat products for food use.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED MEAT, MILK, EGGS AND OTHER ANIMAL FOOD PRODUCTS

Fluid Milk Products. Milk appears to be an appropriate medium for enrichment with iron to help alleviate the deficiency noted in recent surveys. In exploratory research whole milks, with and without prior vacuum "deodorization," were enriched with several ferric ( $Fe^{+3}$ ) and ferrous ( $Fe^{+2}$ ) iron compounds before pasteurization at levels of 10 to 40 mg. per quart. Milks containing 10 mg of Fe per quart which were "deodorized" before adding the iron (except those enriched with

$\text{Fe}^{+2}$  fumarate) were acceptable beverages. Without deodorization the addition of ferric compounds resulted in lipolytic rancidity even after pasteurization at temperatures up to 168°F. for 16 seconds.

The ferric-activated rancidity diminished with increasing pasteurization temperature within the range of 162-170°F. Ferrous compounds did not cause lipolytic rancidity. A slight oxidized flavor was detected in some samples, but the oxidized flavor was more noticeable in samples enriched with ferrous compounds.

Studies of chemical changes in stored sterile milks resulted in the identification in stale sterile concentrated skim milk of several highly flavorful compounds not previously reported. These include 6-trans-nonenal, methional, 2-phenylethanol, skatole, cinnamaldehyde and ethyl cinnamate. It was also shown that vanillin is present in sterile concentrated milks at levels below its reported flavor threshold.

In other flavor research over forty new compounds were isolated and identified from a dichloromethane extract of a lactose-casein browning system. They consist of fifteen C-5 to C-14 furanics, five pyrazines, three pyrroles, three lactones, 2-acetyl pyridine, acrolein, cyclopentanone, 2-methyltetrahydrofuran-3-one, acetoin, acetophenone, propiophenone, benzaldehyde, benzyl alcohol, phenol, N-methyl-2-pyrrolidinone, isopropylidene glycerol, acetol and acetol acetate. Only six of these compounds have been previously identified in milk or milk-related systems. The study of lactose-casein browning, along with flavor evaluation of the products identified, should shed light on the chemistry of milk products deterioration and off-flavor development.

Contract research at the University of Maryland has shown that the level of fat in the milk and fatty acid components in the glycerides will vary with the type of ration. For example, results indicate that, if corn silage is fed to the cows and the concentrates are limited, a more saturated milk fat could be produced. When problems occur in the processing of milk, feeding programs of the source dairy should be checked, with the thought that feeding high energy levels, and thus large amount of cereal grains, contributes to production of high level of unsaturated fatty acids in milk fat. Since the economy of feeding large amounts of grain has been stressed only recently and slowly adopted, this trend may be the cause of some of the problems with fat stability in processing market milks.

A process for the manufacture of a stable whipping cream preserved by high temperature short time sterilization is being investigated. This

work involves manipulation of fat content, homogenization pressure, heat treatment and addition of stabilizers and emulsifiers. Creams containing 32% butter fat (b.f.) with certain combinations of carrageenin, carboxymethylcellulose and algin stabilizers have remained fluid and whippable for 3 to 4 months at 70°F. Added milk solids-not-fat aided in the prevention of serum separation. Creaming has been substantially reduced by the addition of stabilizers to creams containing 32% b.f., but still is a major problem in the low fat products which are much less viscous.

Casein of human milk may be presumed to represent the ideal to be approximated for human feeding by the casein of the cow or other animal. Research has revealed that the major human casein is a single protein, more like cow  $\beta$ -casein than cow  $\alpha_{s1}$ -casein. The human casein is phosphorylated at several levels, precipitated by  $\text{Ca}^{++}$  and stabilized by a human casein fraction similar to  $\kappa$ -casein of cow milk.

In research conducted under a PL-480 grant at Indian Institute of Science, Bangalore, Indian water buffalo casein has been shown to consist of at least four major components,  $\alpha_s$ -,  $\beta$ -,  $\gamma$ - and  $\kappa$ -caseins, similar, but not identical to the components of cow casein. An outstanding difference in composition is a markedly higher content of cystine in buffalo  $\kappa$ -casein. Polymorphism of buffalo  $\beta$ -casein was demonstrated at EU.

Evidence is presented indicating that phosphorylation of protein to form casein in the mammary gland can, to some degree, take place before synthesis of the entire peptide chains. This interpretation is contrary to the generally accepted view that phosphorylation occurs after synthesis of the complete polypeptide chain.

Studies at EU have shown that  $\beta$ -lactoglobulin inhibits the phosphorylation of caseins by phosphoprotein phosphatase.  $\beta$ -Lactoglobulins prepared from cow, goat and sow milk all exhibited this inhibitory effect and suggest that  $\beta$ -lactoglobulin may have a reverse role in the phosphorylation of casein proteins. Lipid occurs in multiple crystallized  $\beta$ -lactoglobulin.

The minor casein components,  $\alpha_{s3}$ - and  $\alpha_{s4}$ -casein have been isolated at EU and characterized in terms of their phosphorus content, calcium sensitivity, and amino acid composition. These proteins contain sulphydryl groups that may be factors affecting heat stability and off-flavor development in milk.

Determination of electric birefringence properties of  $\alpha_{s1}$ - and  $\kappa$ -caseins has provided information regarding the structure and electric properties of the casein aggregates, facilitating the elucidation of the mechanism of micelle stability.

A simple, rapid and specific colorimetric test for the quantitative determination of free sulfhydryl groups in milk and dairy products was developed in PL-480 sponsored research at National Dairy Research Institute, Karnal, Punjab, India. A comprehensive study of factors influencing the presence of the sulfhydryl group was carried out. It was found that sulfhydryl content was highest in cow milk, intermediate in buffalo milk, and lowest in goat milk. Breed had little effect on sulfhydryl content of cow and buffalo milk. Sulfhydryl content was highest immediately after start of lactation and when fresh forage was available. The addition of cysteine to sweetened condensed milk from buffalo milk significantly increased storage life. Storage life of dehydrated dairy products was affected more by moisture content than by sulfhydryl content. The green cheeses made from buffalo milk were relatively low in sulfhydryl content in comparison to those foods from cow milk. This difference narrowed during storage, and the sulfhydryl content in cheese increased at the expense of the disulfides during the first month of storage; thereafter the trend reversed.

The A protein component of lactose synthetase was isolated and studied under a grant at Oklahoma State University, Stillwater. The A protein contains 8-10% carbohydrate, has low content of acidic amino acids, may have multiple forms, and appears to transfer galactosyl residues to N-acetylglucosamine except in the presence of  $\alpha$ -lactalbumin, when glucose acts as the acceptor and lactose is formed.

Small-angle X-ray scattering research at EU has given radii of gyration and other solution parameters for lysozyme,  $\alpha$ -lactalbumin, ribonuclease,  $\beta$ -lactoglobulin and a riboflavin-binding transfer protein. In PL-480 sponsored research at University of Graz, Austria, small-angle X-ray scattering studies indicate that t-ribonucleic acid is an elongated body with two different cross-sections, up to 40°C. Above this temperature, unfolding occurs. At Israel Institute of Technology, Haifa, the main conclusion of studies on unnatural nucleic acids is that 5-fluourouracil inhibits the biosynthesis of ribosomal proteins.

Self-association studies were carried out on the  $\beta$ -lactoglobulin,  $\alpha$ -chymotrypsin, and microtuble protein systems.  $\alpha$ -Chymotrypsin dimerizes as a result of ion-pair formation between His 57 and Tyr 146.  $\beta$ -Lactoglobulin A tetramerization is driven mostly by hydrophobic interactions; it is preceded by a conformation change with the uptake of 16 protons per aggregate formed and the forced formation of 16 carboxyl-carboxyl hydrogen bonds. Studies on interactions of proteins with solvent components showed that bovine serum albumin interacts preferentially with water in mixtures containing methoxyethanol.

Sterile milks could be obtained with lower processing temperatures if all microbial forms present were heat-labile. Enzymes, e.g., spore disulfide reductase, functionally involved in germination of heat resistant bacterial spores continue under study at EU. A fraction of spore extract from Bacillus cereus T has been shown to stimulate the enzymatic activity of spore lytic enzyme which is believed to break down spore cortex during germination. In related studies at U. P. Agricultural University, Pantnagar, India, under a PL-480 grant, the addition of picolinic acid hydrazide lead to formation of heat labile spores, while diethyl dipicolinate inhibited sporulation of B. cereus T without affecting growth.

Dry Milk Products. Pilot plant development of both EU processes (spray foam and vacuum foam) for drying whole milk is substantially complete. In further development of the continuous vacuum process for foam-drying whole milk, it was shown that the agitated falling film vacuum evaporator will operate for sufficient hours, before cleaning becomes necessary, to be commercially practicable. Likewise, a doctor blade system was designed, built and tested that will permit continuous dryer operation for periods sufficient for commercial practicability in the production of dry whole milk.

The preparation of dehydrated reconstructed milks at EU has among its objectives obtaining a product with flavor stability at room temperature. A method was worked out for separating the volatile materials from stale milk fat into ketone, lactone and free fatty acid fractions. The contribution of these three fractions to stale flavor in products was studied. Preliminary results indicate the lactone fraction is the most important. Steam distillation conditions for removal of various amounts of volatile materials and their precursors from milk fat were also determined. Complete removal resulted in bland milk fat equal in flavor properties to coconut oil. This material does not contribute any flavor when added to skim milk. Partially deodorized milk fat improved the flavor of skim milk to which it was added. The desirable flavor characteristics of milk fat are attributed primarily to volatile compounds which probably are unique to this fat.

Powders made from milk into which partially deodorized milk fat was homogenized before condensing and drying showed excellent flavor stability during storage at room temperature.

In complementary contract research at Pennsylvania State University, University Park, the delta-lactones (which are generated in milk fat from hydroxyglycerides) were shown to actually be intermediates in a new metabolic pathway of fatty acids. This pathway, (denoted as delta-oxidation) apparently exists in many mammalian tissues and points out

the unique importance of flavor precursors and their relationship to milk fat synthesis. Radioactive isotope tracer techniques revealed that acetate is the common precursor of both the hydroxy and saturated acids produced in the secretory cells of the mammary gland. This indicates that genetically controlled metabolic variations might exist which would lead to low lactone precursor production in milk fat. A positive correlation was found between the lactone and methyl ketone producing capacity of milk fat. Therefore, if animals can be found (or bred) that produce low levels of lactone precursor in their milks, products (especially whole milk powder) made from that milk should undergo little or no nonoxidative flavor changes in the fat phase during processing and storage.

Incorporation of antioxidants in whole milk powders is being studied at EU. Results of storage stability tests indicate that a mixture of methyl gallate and butylated hydroxyanisole affords some protection against oxidation.

In a part of the continuing investigation of components of dairy products related to flavor, a method for the quantitative determination of glyceryl-1-ethers was developed. The ethers were isolated and partly characterized. In milk fat the total amount is about  $0.25 \mu$  moles/g. Concentration of glyceryl ethers in colostrum lipids is several fold higher than in normal milk lipids.

In other supporting research it was found that the proportion of water bound to micellar casein in milk is related directly to the heat stability of the milk. If this is a cause-effect relationship, the mechanism is still unexplained. Electrophoresis in agarose-acrylamide gels is useful in discovering quantitatively the association of various kinds of casein-molecules in mixtures.

Investigation of casein micelle formation in lactating mammary tissue has led to the postulation that a macromolecule containing one  $\alpha$ , two  $\beta$ , and three  $\alpha_{s1}$ -casein molecules is synthesized on the ribosome and passes to the Golgi vacuole where carbohydrate, phosphorylation and calcium incorporation result in cross-linking the proteins and formation of a stable casein micelle. This hypothesis offers an explanation for the presence of casein components in constant ratios; structural stability; and sensitivity to rennin and divalent ions.

The close genetic linkage in the biosynthesis of  $\beta$ - and  $\gamma$ -caseins, discovered by disc-gel electrophoretic typing and supported by evidence from amino acid analysis, has been verified further by fingerprinting of chymotryptic peptides and by terminal amino acid analysis. Peptide maps of related  $\beta$ - and  $\gamma$ -caseins have strong similarities. Polymorphs

of both caseins all have arginine as N-terminal and the sequence -isoleucyl-isoleucyl-valine as C-terminal. Other minor components of casein micelles have been isolated in the course of preparing pure  $\gamma$ -caseins; these have been named TS (temperature-sensitive), R and S caseins. TS casein occurs in electrophoretically different forms. In amino acid composition R, S and TS caseins are similar. Like other caseins they contain much proline, glutamic acid, valine and leucine, but no cystine. Evidence at hand suggests that at least one, and perhaps two, polymorphic proteins, genetically controlled, make up this family of newly isolated caseins.

In further research on dehydrated milk products it was found that homogenizing nonfat milk before drying can produce a powder which can be whipped to a stable foam after reconstitution. Milk protein degradation during processing decreases the nutritive value of milk as evidenced by a decrease in the quantity of available lysine. Attempts to measure the amount of available lysine in pure proteins and food products containing milk proteins by a new method using 2,4,6-trinitrobenzenesulfonic acid (TNBS) were only partly successful due to the interference of glycoproteins (e.g.  $\kappa$ -casein) present in milk.

Milk Fat. To improve the market for milk fat by development of new forms for consumer use, a continuous method for the manufacture of anhydrous milk fat is being developed. An improvised system has been built which provides for rapid heating and holding of the fat. Studies presently underway will determine what period of holding produces the most desirable flavor in the product. Concurrent studies on hydrogenation of unsaturated fatty acids in milk fat have shown that selective hydrogenation is difficult to achieve, although some preferential hydrogenation has been accomplished. Milk fat has been separated into several fractions by a zone melting procedure. By selective adsorption under carefully controlled conditions, the cholesterol content of milk fat has been reduced 25%. Deodorization of milk fat for use in reconstituted milk products is described under Dried Milk Products.

Research at the National Dairy Research Institute, Karnal, Punjab, India, under a PL-480 grant has shown that whether the milk fat employed in the manufacture of ghee is obtained from cow or buffalo has little influence on the final flavor of the product. However, organoleptic evaluation of various samples have established that significant differences exist in the flavors of ghee prepared by the Direct Creamery and the Desi methods. The temperature of clarification of cream/butter also has a significant impact on the ultimate flavor of ghee.

Cheese. Excellent quality low-fat cheese has been consistently manufactured on a pilot plant scale. The cheese cured for 6-12 weeks was

packed in consumer sized packages and dispensed in four stores of a local food chain through a cooperative project with the Economic Research Service. Results indicate that this type of cheese was accepted and is marketable. Complete details of the study have been published by Economic Research Service, USDA.

Processed cheese prepared from low-fat cheese has good storage properties. Commercial cheese companies are interested in this new product.

The biochemical characteristics of commercial and freshly isolated lactic acid bacteria were investigated at National Dairy Research Institute, Karnal, in PL-480 sponsored research concerned with understanding and improving the flavor of dahi, yoghurt, acidophilus milk and cheeses. A total of 90 Streptococcus and 35 Lactobacillus strains were isolated and identified by morphological, biochemical and cultural characteristics. Representative strains were studied in detail with respect to the production of lactic acid, volatile acids, acetoin, diacetyl, acetaldehyde, proteases and lipases. Highly active strains were used in the preparation of several lots of dahi and yoghurt and then tested organoleptically. Biochemical activity of selected bacterial strains were not improved by use of mutagenic agents.

#### FOREIGN MARKET DEVELOPMENT

Cheese Whey Blends. A whey-soy powder containing approximately 50% of its lactose in enzymatically hydrolyzed form has been made and submitted to Human Nutrition Division for evaluation. Such proteinaceous products are sought for food uses, especially to improve the nutritional level of people in developing countries. In supporting research, the water binding properties of soy-whey powders were studied using gravimetric methods and differential scanning calorimetry. This was done in an attempt to obtain data leading to the resolution of difficulties encountered when attempting to spray dry mixtures of Cottage cheese whey and soy stabilized by addition of gums. The gums have been found necessary to keep the soy protein in suspension at the pH of Cottage cheese whey.

Factors influencing the crystallization rate of lactose in condensed wheys have been investigated and whey powders have been produced containing high levels of crystalline lactose using an essentially single pass operation.

A high protein powder to be reconstituted with water for beverage use was made from a base consisting of a spray dried mixture of milk and eggs. This base is dry blended with sugar, cocoa, vitamins and minerals to produce a powder whose flavor stability during storage is under investigation.

A method for producing a powder containing 80% whey protein includes the use of polyphosphate to precipitate the whey proteins and their subsequent collection by continuous centrifugation. The protein sludge is solubilized by neutralization with base and the resultant protein solution is reduced in salt and lactose content by passage over Sephadex. The partially desalted solution is condensed and spray dried in conventional fashion. The product has good solubility.

#### PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISM AND NATURALLY OCCURRING TOXINS

Salmonella in Cheese. A study of the manufacturing variables in Cottage cheese manufacture led to recommendations of manufacturing conditions which would insure complete destruction of contaminating salmonellae. These include a minimum cooking temperature of 125°F. and conditions which promote good development of acid. Similar studies on Cheddar and Colby cheese indicated that many of the manufacturing variables had a significant effect on salmonellae survival, but that cells might remain viable in good quality cheese for several months. This is attributed to the lower temperatures and different conditions of acidity of the latter processes. The need for proper sanitation in all phases of operations is emphasized.

New and improved biochemical methods for the detection of salmonellae were investigated and a 24-hour cultural presumptive-type test was developed. The immunofluorescent antibody technique was applied in the detection of salmonellae in nonfat dry milk.

Milk Allergens. Studies were continued on the development of new antigenic specificities derived from purified milk proteins during brief enzymic digestion. These observations may explain why some milk allergy patients do not exhibit skin reactions to milk. They may be allergic to the digestive products of milk. Data on the incidence of positive skin reactions to cow's milk and identification of reagins (allergic antibodies) specific for the individual proteins of milk have been collected. The results will provide an estimate of the incidence of allergy to milk in the general population, and identify the principal allergenic proteins of milk.  $\alpha$ -Lactalbumin and  $\beta$ -lactoglobulin reacted more strongly and in significantly higher dilutions than other milk proteins. Only mild to moderate reactions were observed with the casein fractions,  $\gamma$ -globulin, and mucoprotein and these reactions could be largely accounted for by the contaminants detected in them.

Three immunoglobulin A (IgA) proteins have been discovered in bovine body fluids. The quantitative study on the levels of immunoglobulins in the body fluids of the cow is the first of its type and will provide important reference values for IgG1, IgG2, IgA and IgM before, during, and following calving. The ratio of IgG:IgA ranged from

1000:1 in serum to 1:1 in tears and saliva. The demonstration that IgA is a major component of tears and saliva is in agreement with the pattern described for other species. This suggests that IgA is synthesized locally and is probably a very important antibody against viral and bacterial pathogens that enter via the digestive, respiratory, urogenital and mammary systems.

Research directed toward development of specific sensitivity in experimental animals corresponding to allergy in the human has been successful in one species--the rat; and partially successful in a second species--the rabbit. Results in rabbits lack the uniformity required for an assay method.

#### ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

Whey Disposal by Utilization. Pilot-plant scale studies with reverse osmosis equipment indicates the feasibility and commercial potential of this method for the concentration of whey. Commercial equipment manufacturers are actively developing equipment for this application.

Detailed research on the fractionation of whey by reverse osmosis has been completed. A concentrate of essentially the same composition of skim milk has been produced by partial removal of ash, lactose and water.

Studies on the problems and methods of control of microbial contamination of the equipment used in reverse osmosis have led to suggestions for changes in design and selection of sanitizing agents.

Unique fractions of whey in which the protein has not been denatured provide a basis for future research on their chemical and physical characterization and their utilization in food products. In supporting research, "true" protein and polypeptide content, and the amino acid composition of both were measured for the principal types of cheese whey.

Milk whey contains a component which releases a kinin-like peptide upon trypsin digestion. This component has been isolated in very pure form and the physiological characteristics are being studied.

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## MEAT UTILIZATION

### Problems and Objectives

Meat of improved stability, including enhanced retention of acceptable flavor and color characteristics, is needed so that more efficient processing and packaging in centralized locations with resultant economies is possible. Processed meat products, two-thirds of our pork and significant quantities of other meats, need better keeping quality relative to color and flavor retention in distribution channels. Processes need to be evaluated to prevent introduction of potential chemical health hazards into cured meat products. Technical advances are needed to enable the numerous small meat processors to manufacture better products. Contamination of meat with pathogenic microorganisms is a serious problem which must be eliminated through improved processing techniques.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Provide procedures for the preparation of meat and meat products with initially desirable organoleptic and physical characteristics which are retained in storage.
2. Provide technology which can be applied to the problems of small-scale processors and improve the meat products of rural industry.
3. Improve meat processing methods so that the consumer can be assured of safety in meat and its products.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED MEAT, MILK, EGGS AND OTHER ANIMAL FOOD PRODUCTS

Lamb and Mutton Products. New lamb products were prepared and evaluated in research conducted under contract at Pennsylvania State University, University Park, Pa. The products tested included smoked lamb sausage, lamb loaf, lamb curry prepared as a "boil-in-bag" product, barbecued ribs, boned lamb shanks in sauce, stuffed lamb breast, and marinated lamb for shishkebab. Products were served in the University cafeteria. The lamb curry, lamb sausage, lamb loaf, and meat for shishkebabs were also demonstrated to two large industrial firms who are potential producers of these products. The new products can be produced from lower priced cuts and by employing selected conventional procedures.

Cooking Processes. In contract research at Cornell University, Ithaca, N.Y., studies of institutional cookery of meat were completed. The greatest tenderization of meat during cooking was shown to occur in the 50-60°C. (122-140°F.) range. Long-time low-temperature cooking (below 60°C.) was shown to tenderize meat more than faster heating owing to collagenolytic activity persisting in the low temperature cooked meat.

Heat resistant microorganisms in canned meats are being studied under a PL-480 grant at University of Veterinary Medicine, Vienna, Austria. Results to date indicate that either propyl gallate or lauryl sulfate can materially reduce the thermal resistance of micrococci which often survive heat treatment in canned meat products. This finding may lead to a new concept of thermal processing of non-sterile canned meats.

Process Development and Control. Data was obtained at EU on the effects of types and levels of fat and rates and temperatures of comminution on the processing and characteristics of frankfurters. Optimum conditions were found to be time as well as temperature dependent. Fat separation during processing of frankfurters seems to be a function of fat melting point, high melting fat resulting in more separation. Shrinkage was inversely related to fat content. The air content of frankfurters varied inversely with the maximum temperature attained during comminution. Frankfurter skin strength was lessened on increasing the temperatures of comminution; elasticity, the equivalent of rubberiness, decreased under these conditions. This study has potential application in providing meat processors with information for improving the quality of meat emulsions and that of the meat products, such as frankfurters and bologna, prepared from them.

In research to develop rapid analytical methods for process and product control, an intensive study of the official (AOAC) method of fat determination for meats was undertaken. Under certain conditions, the 7-1/4 hours (minimum time) procedure could be reduced to less than 2 hours for 15-60% fat levels of ground meat or emulsion (hamburger, pork sausage and frankfurter preparations), resulting in a recovery of 94-100% of the fat level obtained by the official method. The savings in time and cost of equipment would be especially beneficial to the smaller processor who cannot hold over meat mixtures while awaiting results for fat analysis. Although individual species fats show a linear relationship between refractive index and total extractable fat, refractive index could not be used as a measure of total fat content of mixed trimmings.

At the Veterinary Research Institute, Pulawy, Poland, in PL-480 sponsored research, a large number of strains of microorganisms have been isolated from cured meat and their physiological characteristics examined. Eighty-nine percent of the strains were micrococci. Antagonistic actions between isolated bacteria were noted which may prove of importance and significance to meat technology. Curing salts increased antagonistic activities.

Meat Color. As a result of research at EU to improve the color of meat, a complete scheme for the reaction between hydrogen peroxide and the pigments of meat has been developed. Thirteen reaction steps have been observed or deduced mathematically from the kinetics of the conversion of the brown reaction product, metmyoglobin, through two intermediates to the products hydroperoxymetmyoglobin (green, mild acid conditions) and peroxymetmyoglobin (red, mild alkali). Investigation of the relation of these pigments to discolorations in meats has begun by adding hydrogen peroxide along with sodium sulfide and ascorbate, with and without oxygen, to ground meat mixes, meat emulsions and extracts. The reaction is variable under any given set of conditions, and is dependent on the form of meat used, the relative concentrations of the reactants, and the rate of addition. A cell designed to determine absorption spectra in turbid materials was developed for these spectrophotometric studies.

Meat Smoking. Ethyl acetate was shown to be a suitable solvent for isolating and concentrating the flavor components of liquid smoke mixtures and appears to offer commercial possibilities for improving smoke concentrates. Partition ratios of phenolic smoke constituents between different oils and water were determined. This information is being used to predict which smoke flavor constituents are most likely to dissolve in several meat constituents. Further studies on composition of smoke preparations and concentrates have led to identification of several more components. A number of fractions believed to contain pure chemicals are now known to be mixtures of two or more components and their contribution to smoke flavor cannot be evaluated until they are separated.

Meat Flavor. Since curing seems to convert the flavor of all meats to a somewhat constant end product, the effect of curing substances on flavor precursors is being studied. Addition of NaCl changes free amino acid balance and tends to increase content of nine basic amino acids. Nitrite has a somewhat greater effect in decreasing content of free amino acids. Evidently other, as yet undisclosed, factors are responsible for flavor changes on curing.

Gas chromatographic separations of volatiles from pyrolyzed (250°C) meat fractions continues. Tentative identification of five pyrazines was made for the first time in meat. Pyrolysis patterns of cured and

uncured pork diffusates varied primarily in intensity. Pyrolysis patterns of stearic and oleic acids and their glycerides are qualitatively similar and are produced in the presence of oxygen only. Thus, pork, beef, and lamb fat, as well as several classes of lipids from pork fat, gave similar pyrolysis profiles. Porky aroma on heating pork fat is due to water-soluble components.

In grant research at Rutgers University, New Brunswick, N.J., a deterioration of boiled beef flavor during freeze-drying and storage under nitrogen was noted. This was characterized not by the formation of new compounds, but by a change in the relative proportions of the original flavor compounds. The precursors of desirable boiled beef flavor are present in trace amounts. The method developed for isolation and analysis of volatile flavor compounds is reproducible within 5%. Changes observed have been far beyond that range. This method is therefore suitable for analysis of deterioration of meat flavor.

Meat Texture. Myosin is the most important of the meat fibrillar proteins. A more complete understanding of its activity as enzyme and as a polymer is necessary to solving the meat tenderness problem and many problems of binding in meat products. A new method for column chromatography of myosin on hydroxyapatite was developed which promises to greatly expedite other studies of the physical chemistry of myosin. Using a magnetic densimeter, new and more accurate values have been obtained for the specific volume of myosin. Electrophoresis of myosin at low ionic strength has been achieved through the use of pyrophosphate buffer. Preliminary evidence indicates that myosin as usually prepared is heterogeneous and that two physically distinguishable fractions can be separated by electrophoresis. These studies provide basic information needed for the development of improved meat products.

Stability of Meat Products. Present research emphasizes changes in fatty components because of the importance of fat breakdown on flavor of stored meat products. Using a model system to reduce complications, the pro-oxidant effects of metals on meat triglycerides have been investigated. Water-soluble ferrous ion is a very powerful pro-oxidant and could not be readily inhibited by chelators. Metal ions decompose the unsaturated methyl ester hydroperoxides to give unsaturated aldehydes while spontaneous decomposition under acid conditions give largely saturated aldehydes. The differences observed in the aldehydes produced by different oxidizers of methyl oleate hydroperoxide support the hypothesis that there are selective mechanisms acting in hydroperoxide decomposition. This theory, if confirmed, may explain why unequal quantities of monocarbonyl compounds are formed under different conditions of oxidation.

A method has been developed using oxidized methyl oleate to give a rapid (3 hr) indication of the effect of microorganisms on carbonyls and peroxides in rancid fat. This can serve as a screening mechanism for determining microorganisms that have an effect on the oxidation of fats. Out of 14 species of both gram-positive and gram-negative bacteria from nine genera, five were found to have considerable antioxidant activity. They were Micrococcus freudenreichii, Sarcina lutea, Escherichia coli, Bacillus cereus, and Leuconostoc dextranicum. The antioxidant substance is being isolated and studied chemically to learn more about its possible usefulness. When slightly rancid fat was added to ground meat it did not delay the onset of spoilage, as measured by several other parameters, thus showing that rancid fat had little or no bacteriostatic activity in a meat substrate.

We have now demonstrated antioxidant, pro-oxidant, and lipolytic activity in various organisms. The results reported above on antioxidant cell extracts are particularly interesting. There appears to be no special relationship between those cells producing antioxidants and those which are strong hydroperoxide decomposers.

#### PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISM AND NATURALLY OCCURRING TOXINS

Eliminating Salmonellae from Meat. Preliminary results of research at EU indicate that resistance to drying may be the most important factor in the prevalence of any particular Salmonella serotype as a food poisoning organism. This is of extreme importance in evaluating the likelihood of a serotype surviving in a meat processing plant or on meat processing equipment. Survival of 26 serotypes of Salmonella in a frozen ground pork film dried on a stainless steel surface was studied. On dry surfaces there was a sharp division with 12 serotypes dying out within 2 days and the others surviving over 2 weeks. The more resistant strains were the strains most often associated with salmonellae outbreaks. In other studies, the mechanisms of action of the two most commonly used enrichment media for salmonellae, selenite-cystine and tetrathionate broths, are being critically examined. When small amounts of thiosulfate are added to selenite-cystine broth, seleno polythionates are formed. As the concentrations of thiosulfate and selenite plus cystine are increased, the medium becomes progressively bactericidal for Escherichia, Shigella, Arizona, Enterobacter, Klebsiella, Salmonella, Proteus, and Psuedomonas. Tetrathionate broth is bactericidal only to growing cells. This toxicity is not caused by tetrathionate or any other polythionate formed when thiosulfate and iodine are mixed. It is directly related, however, to a critical thiosulfate-tetrathionate ratio in the final broth.

In preliminary results from collaborative studies organized by the International Committee on Microbiological Specifications for Foods, it appears that tetrathionate at 43°C. and Selenite Brilliant Green Sulfa at 43° and 35° are the most efficient enrichment media for salmonellae detection.

Utilizing Microbial Antagonism. Contract research at the University of California (Davis) has shown that any meat product can be rendered free of proclivities for causing food poisoning if it can be processed so as to contain about one percent glucose and  $10^9$  radiation killed cells of Pediococcus cerevisiae. Subsequent to the development of a method for utilizing radiation killed cells of Pediococcus cerevisiae for acid production in meat products as a means of inhibiting undesirable bacteria, attention was given to the isolation of inhibitory members of the indigenous flora of meats. Cultures selected were further screened for acid production, catalase production, and morphology. Five cultures were found which surpassed P. cerevisiae in acid production. Three of these showed marked potential for use as radiation killed cells.

Enterotoxin Production and Assay. Results of research conducted at EU indicate that enterotoxin production by Staphylococci is under some genetic control that is independent of the determinants for all growth. Precautions were taken to insure that clones studied were derived from single cells. A large percentage of the clones producing toxin at titer levels from 2-128 have remained stable for a given titer for 9 months when transferred from one agar slant to another at monthly intervals. The use of microorganisms and higher forms of life to detect harmful substances in food was studied under a PL-480 grant at Central Institute for Nutrition and Food, Zeist, Netherlands. Although painstaking research with insects, fish, fish larvae, yeasts, frog spermatozoa, and gull spermatozoa was carried out, no simple assay system for bacterial enterotoxins has been discovered. In recent studies, certain tissue culture lines do show responses to microbial toxins which might be utilizable for a simply conducted test for the presence of the toxins.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED MEAT, MILK, EGGS AND OTHER ANIMAL FOOD PRODUCTS

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#### PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISM AND NATURALLY OCCURRING TOXINS

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## ANIMAL FATS AND OILS UTILIZATION

### Problems and Objectives

Finding outlets for inedible fats has been a problem ever since synthetic detergents began to replace soap. The problem has been complicated further by the increase of fat production associated with the continuing increase in meat consumption. Previous utilization research activities have been effective in providing markets for over one-fourth of the 4.7 billion pounds of inedible fat that is produced per year. In order to maintain current markets and provide for new and economical ones for this byproduct of the meat industry a strong and progressive research program is necessary. A promising area that is accounting for the use of an increasing percentage of the inedible fat production is that of chemical derivatives. Continued research is needed to provide the chemical industry with new products and processes. In the area of pollution, tallow-based detergents and lime soap dispersing agents, such as sulfated alkanolamides, are easily biodegradable under both aerobic and anaerobic conditions and hence do not persist in the environment. Although the role of phosphates in eutrophication requires clarification, the development of tallow-based phosphate-free detergent formulations is warranted. At the same time, basic and exploratory research on the organic and physical chemistry of fats and fatty acids is needed in order to maintain a continuing supply of fundamental knowledge; to establish new principles and concepts; and to discover new reactions and processes for future development.

Major objectives of the research are to develop:

1. Fundamental knowledge of the composition, structure, and physical and chemical properties of inedible fat and its components.
2. New and improved techniques for separating and characterizing the components and derivatives of fat.
3. New chemical intermediates.
4. New and improved industrial products--particularly for use as biodegradable detergents, plastic foams and coatings and lubricant components and additives.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

Polymers. Improved procedures were developed for preparation of hypo-halogenated glycerides from fats for use in fire-resistant urethane

foams. Direct addition of gaseous hydrochloric or hydrobromic acid to epoxidized lard or tallow yielded halogen-containing polyols suitable for urethane foams. Purification of the crude derivatives by extraction with acetone led to removal of unreacted saturated materials. The purified polyols furnished fire-retardant foams with compressive strengths superior to those previously reported.

Investigations to provide polymers of improved properties were continued. In some copolymers the superior plasticizing efficiency of fatty comonomers was limited, at high fatty content, by crystallization of the long side chains. The lowering of copolymer glass-transition temperatures (taken as index of internal plasticizing) increased linearly with increasing content of fatty monomer as long as such systems remained amorphous. But beyond about 40% fatty component, apparent glass temperature was affected by crystallization ascribed to fatty side chains. There are indications that in three-monomer systems, fatty monomers should give effective plasticization without impairment by side-chain crystallinity. Vinyl chloride terpolymerized with vinyl stearate and vinyl acetate was stabilized against aging by the stearate component.

Isopropenyl Esters. The potential of isopropenyl esters as reactive intermediates was further demonstrated by their application to the preparation of detergents in high yields and free of salt byproducts (see section on detergents). Isopropenyl stearate has been synthesized directly from methylacetylene (propyne) and stearic acid in a new moderate-pressure process. A pressure unit was designed and built for scaled-up preparations of isopropenyl esters in amounts of one gallon per run.

Engineering studies on a laboratory scale of the reaction between isopropenyl acetate and stearic acid to form isopropenyl stearate have been completed. An empirical equation which describes the reaction kinetics has been developed. It was found that the equilibrium constant of the reaction is independent of temperature. Reaction time was reduced from 5 hours to about 10 minutes. Physical property data required for designing a continuous process have been determined, a feasible recovery scheme has been developed, and a bench scale continuous process line is being designed. The propyne-stearic acid process will also receive kinetic and engineering study. When this work is completed it will be possible to choose the reaction on which to base the ultimate process.

$\alpha$ -Anion Procedure. A new reaction has been developed for substituting straight chain and  $\alpha$ -branched fatty acids at the  $\alpha$ -position; substituted malonic acids and di- or tri-alkylacetic acids were made in nearly quantitative yields. The mechanism of  $\alpha$ -anion formation from  $\alpha$ -branched acids was studied by deuteration and carboxylation techniques resulting in 95% conversions to  $\alpha$ -anions. Straight chain fatty acids were

converted in one step to aldehydes and nitroalkanes by the new  $\alpha$ -anion procedure. The procedure appears to be a significant development because of the versatility of the reaction.

Animal Lipids. Study of the lipid composition of light and dark pork muscles showed the former to be 20% richer in lipids. The major components were glycerides and phospholipids, dark muscle being relatively richer in the latter. The lipids contained 60% unsaturated fatty acids, including 15% linoleic, an essential fatty acid. This finding, in conflict with commonly held ideas of animal fat composition, is of great importance to the fields of nutrition, diet and health.

In supporting research, the derivation of equations reliably predicting solute output profiles in countercurrent distribution, under conditions of two-phase flow, is a significant contribution to separations science. Widely-used ideal equations for countercurrent distribution were shown to be inaccurate when two-phase flow occurs. Since this is a most common case, equations were derived that apply more reliably to real experimental conditions for both frontal and single-withdrawal operations. Yielding greater quantities of product at comparable purity, the frontal technique is preferable in preparative work.

Methods. In a study coordinated by EU, collaborators of the AOCS Smalley Gas Chromatography Check Program determined the composition of 2 methyl esters mixtures and 13 oils. The results were treated statistically. They indicated the relative difficulty in analyzing the various oils and the equal precision afforded by thermal conductivity and flame ionization detectors. The results permit appraisal of GLC-based data and indicate procedures needed for highest accuracy.

Triglyceride Synthesis. Under a grant to the University of Connecticut, Storrs, methods for the synthesis and purification of triglycerides have been developed. Grantee synthesized and furnished 40 glycerides of very high purity and varied acyl units to EU; four were enantiomeric. Low temperature solvent crystallization techniques were also developed for bulk purification of the glyceride reaction products and a new chromatographic alumina-column treatment was perfected as a final purification step. Laboratory synthesis of high purity glycerides demonstrates the possibility of these glycerides being produced in comparable purity by commercial sources. Eventually, these important compounds would be available to all scientists for research in the development of products of specific nutritional or desirable physical properties. At the present, the methodology for preparing mixed acid triglycerides of high purity should be valuable to firms manufacturing GLC and TLC standards.

Fatty Acid Products. A practical method for improving the color and odor stability of commercial oleic acid has been developed in research

conducted under a grant at Rutgers State University, New Brunswick, N. J. Color and odor deterioration found in fatty acids derived from commercially produced tallow was traced in part to raw material deficiencies, in part to damage in processing. Laboratory experiments showed the feasibility of a new process for manufacture of fatty acids with improved stability.

Lubricants. Developmental tests have been carried out on the suitability of lithium epiminostearate as a multipurpose grease thickener and are encouraging. Preparation of a one-pound sample of the material for further testing is essentially complete. Lithium salts of related epimino fatty acids were found to have unexpectedly high water solubility together with excellent rust inhibiting capability.

Efforts are continuing to develop methods of aziridine synthesis which are economically more attractive than those presently available. The reactions of model aziridines, fatty aziridines and benzenesulfonamido aziridines with various nucleophilic reagents were studied with the view of preparing derivatives having improved wear characteristics in lubricant applications. Ring-opening reagents employed successfully included ammonium thiocyanate, thiourea, mercaptobenzoic acid, mercaptoacetic acid, ammonium thiosulfate, p-toluenethiol and others. The iodide catalyzed isomerization of N-acyl aziridines to oxazolines was found to be stereoselective in nature.

Work on the development of new ring systems containing nitrogen and/or sulfur atoms, and on new chain substitution derivatives of fatty acids bearing the same heteroatoms, was continued. Methods were developed for the preparation in good yield of fatty thiazolidone thione derivatives. These cyclic compounds had good extreme pressure and average wear characteristics.

Cyanoethylation of keto fatty acids has been accomplished. This results in the introduction of up to four cyanoethyl groups into fatty ketone molecules, giving these compounds polyfunctionality. The new cyanoethylated ketones are much more stable than the cyanoethyl ethers prepared previously.

A homologous series of fatty acid esters has been prepared and has been tested for rubber swelling properties. An inverse relationship between ester chain length and rubber swell has become apparent. Capability for grease testing and for rubber swell determination have been added to the lubricant testing facilities.

In supporting PL-480 sponsored research at Polytechnical University, Gdansk, Poland, general kinetic equations have been obtained which

describe variations of peroxide content as a function of time in a three-step process, including an auto-catalytic, nonperoxidic activated complex in the induction step. These results provide the means for calculating rate constants, thermodynamic functions (free energy, enthalpy, etc.) and the probability of the duration of the activated complex at each step. These equations are general in nature and their use is not restricted to the autoxidation of the erucate, oleate, linoleate, and linolenate methyl esters studied in this project.

#### ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

Biodegradable Detergents. Tallow-based sulfated alkanolamides which are effective detergents and lime soap dispersing agents were prepared from propylene oxide and hydroxyethylpalmitamide and stearamide, followed by sulfation, to give  $\text{RCOHC}_2\text{H}_4\text{OCH}_2\text{CH}(\text{CH}_3)\text{OSO}_3\text{Na}$ ; or from methyl palmitate or stearate and diglycolamine to give  $\text{RCOHC}_2\text{H}_4\text{OC}_2\text{H}_4\text{OSO}_3\text{Na}$ . These sulfated alkanolamides are easily soluble, stable to hydrolysis and biodegradable under both aerobic and anaerobic conditions. Sulfated alkanolamides from coconut oil were soluble, but were shown to have a high critical micelle concentration and to be too hydrophilic to have useful surface active properties. Tallow derivatives have the correct balance between hydrophilic and hydrophobic properties, and good foaming, detergent and lime soap dispersing properties result.

Surface active agents were made from isopropenyl esters in better yield and purity than by the commercial acid chloride method. The 2-sulfoethyl esters (Igepon T type  $\text{RCO}_2\text{C}_2\text{H}_4\text{SO}_3\text{Na}$ ) and the N-methyltaurides (Igepon T type  $\text{RCO}(\text{CH}_3)\text{C}_2\text{H}_4\text{SO}_3\text{Na}$ ) of pelargonic, lauric, myristic, palmitic, stearic, oleic, phenylstearic and tallow acids were prepared.

A method for following the course of aerobic biodegradation of anionic detergents by automated analysis for C, MBAS (Methylene Blue Active Substance) and sulfate ion showed tallow alcohol sulfates, ether alcohol sulfates,  $\alpha$ -sulfo esters and linear alkylbenzenesulfonate (LAS) to decrease in ease of biodegradation in that order. Completely anaerobic conditions for biodegradation are seldom realized but are approximated by relatively common microaerophilic conditions under which biodegradation occurs at a low oxygen level of about 1 ppm or less. A method based on the river water test was developed for simultaneous measurement under both aerobic and microaerophilic conditions. Tallow alcohol sulfates, sulfated alkanolamides, ether alcohol sulfates,  $\alpha$ -sulfo esters and LAS were examined. Tests were carried out in river water containing 5 or 10 ppm of the detergent at 25° or 35°C. Aerobic and microaerophilic experiments were stirred by a continuous stream of air or nitrogen, respectively. Biodegradation was followed by analysis for MBAS using an

autoanalyzer. Tallow alcohol sulfates and tallow-based sulfated alkanolamides were the most easily degraded under either condition and LAS the most resistant. Preliminary experiments have shown that tallow-based detergent formulations based on soap-syndet-lime soap dispersing agent combinations may require little or no phosphate builder. Tests representing the result of multiple washings are required and are being developed to confirm this.

In supporting research, phenylstearic acid from oleic acid was shown to be a complex mixture of 12 position isomers. It forms oil-soluble soaps and is an intermediate for surface active agents. Isolation of 4 crystalline isomers permitted correction of a distribution curve based on alkyl aryl ketones formed by  $\text{CrO}_3$  oxidation. Experience with the complex nature of oily phenylstearic acid suggests that other addition reactions of oleic acid that result in a mixture of isomers could be investigated in a similar manner, i.e., oxidation followed by GLC (gas-liquid chromatography).

Publications and Patents - USDA and Cooperative Programs

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## HIDES AND LEATHER UTILIZATION

### Problems and Objectives

Finding outlets for hides has been a continuing problem even since synthetic substitute leather-like materials began to replace leather. The problem has been further compounded by the increase of hide production as a result of the continuing increase in the slaughter of cattle. In order to maintain current markets and provide for new and economical ones for this important product of the cattle industry, which accounts for 6-10% of the live weight of the cattle, a strong research program is necessary. Areas in which the current research program seeks to improve the market for hides are in the development of leathers with new and unique properties and in the development of food uses. Another problem to be solved if the leather industry is to survive is the control of pollution resulting from the processing and tanning of hides. Areas of needed research include processes to recover protein and fat from fleshings, trimmings and hair; preservation of hides without the use of salt, and elimination or reduction of content of soluble and insoluble solids in tannery effluents. At the same time a strong basic and exploratory research program is needed in order to maintain a continuing supply of fundamental knowledge regarding the physical and organic chemistry of collagen which is necessary for future developments.

Major objectives of the research are to develop:

1. Fundamental knowledge of composition, structure, and physical and chemical properties of hides and their components.
2. Procedures for attaining high quality raw stock.
3. Pollution control during processing and tanning of hides.
4. New and improved techniques for processing hides into leathers.
5. Chemical modifications of hides that result in the development of leathers with unique and desired characteristics.
6. Edible uses for the collagen of cattle hides.

### Progress - USDA and Cooperative Program

#### NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

Improved Products. Shearlings (sheepskin with wool on) dyed with

reactive (Procion) dyes produced wool with wash-fast pastel colors. Attempts to intensify the colors by various pretreatments did not succeed. Glutaraldehyde tanned shearlings had been previously demonstrated to be washable and dry-cleanable. Interest is now growing for use of dyed shearlings for rugs and clothing.

Shearlings have been shown to have advantages over other materials as hospital bedpads. Related contract research at Southern Research Institute, Birmingham, Alabama, to determine efficiency of disinfectant methods has been completed. Laundering in water alone, or in water and commercial detergents or a commercial detergent-sanitizer caused only slight reductions in viral titer or bacterial count on contaminated bedpads. A quaternary ammonium compound, a phenolic disinfectant and alkalinized glutaraldehyde produced a substantial reduction in vaccinia virus and Staphylococcus aureus but had less effect against the more resistant polio virus and Pseudomonas aeruginosa. The effectiveness of the disinfectants was generally not improved by increasing their concentrations above those recommended by the manufacturer. Glutaraldehyde, even at 0.5% (one fourth the recommended concentration) was the most effective of the disinfectants tested. Glutaraldehyde was equally effective against contamination by direct contact or from aerosols. These results can be used as a basis for recommending procedures for laundering and disinfecting shearlings.

In cooperative research by WU and EU, results from a standardized soiling procedure indicated that the WURLAN process did not significantly improve the launderability of the wool on shearling medical pads, thereby failing to improve these pads for use under incontinent patients.

Gloving leather produced by tanning with glutaraldehyde and coloring with Procion dyes has been found to meet the Quartermaster requirements of perspiration resistance and washability for fliers gloves. Flame resistance can be imparted to gloving leather by impregnation with halogenated phosphate esters.

Catfish skins available from fish-farming and fish skins from a number of other species submitted by an informal collaborator, were tanned using standard procedures. Most skins yielded strong leather, but only dolphin and salmon skins were attractive. Since the cutting value is low, high-fashion uses would have to be found.

In supporting research, a determination of the amino acids in the wool of tanned shearlings showed that only lysine reacts with glutaraldehyde, forming stable intermolecular bridges. The durability of insole leather to perspiration is substantially improved by treatment with glutaraldehyde.

Leather Properties. Comfort properties of shoe leathers were evaluated under PL-480 sponsored research (now completed) at Central Leather Research Institute, Madras, India. Considerable physical data were collected on various types of leathers which indicated that leather has a great capacity to absorb and transmit perspiration in the vapor form while resisting the penetration of liquid water. Leather also has a desirable degree of elasticity and plasticity to conform to the foot yet provide adequate support and a degree of resistance to fungal growth which aids in preserving foot health. Subjective evaluations of various footwear, some containing impermeable barriers to water vapor, showed that the dissipation of heat and moisture play an important role in the comfort qualities of footwear. An index involving temperature and relative humidity was developed which may be capable of predicting the conditions under which shoes will be comfortable or uncomfortable.

Under a PL-480 grant to British Leather Manufacturing Association, Milton Park, England, a number of reactive compounds were investigated for improving the stability of leather to attack from chemicals, microorganisms and heat. The fungistatic action of p-nitrophenol and mercaptobenzothiazole can be enhanced by binding these compounds to collagen through the intermediate derivative made with cyanuric chloride. Work on enhancing the substantivity of leather lubricants demonstrated that long chain amines or alcohols can be added to collagen by reacting with formaldehyde or cyanuric chloride as the respective intermediate. It was found that collagen has insufficient reactive groups to permit binding of enough oil components through these covalent linkages to give adequate lubrication.

Tanning Investigations. The kinetics of the reaction of chrome with hide powder and hide slabs have been studied under conditions which adequately simulate commercial practices and, at the same time, permit accurate control and measurement of such operating variables as temperature and degree of agitation. At the chrome concentrations used in these experiments, the rate-controlling mechanism appears to be diffusion in and through the hide substance. A mathematical relationship which assumes diffusion of reactants (chrome complexes) through the hide accompanied by reaction at specific sites (carboxyl groups) appears to be in good agreement with the experimental data thus far obtained. Additional experiments will be conducted to test the validity of this equation over a wide range of processing conditions. It is hoped that this equation will serve as a guide in the development of improved processing techniques. The atomic absorption spectroscopic method for determining chrome in hide was studied and found to be very reliable. This method of analysis is quicker than the standard method of the American Leather Chemists' Association and will save much time.

Interaction between collagen and mineral tanning agents is being investigated under a PL-480 grant at Central Leather Research Institute, Madras, India. Isotopic exchange studies employing sulfate ions containing S<sup>35</sup> were carried out with three isolated chromium sulfate complexes. Ionic sulfate was determined by precipitating as benzidine sulfate. A method to estimate complexly bound sulfate was developed. The effect of chrome concentration, temperature, basicity and added salts on the rates of exchange of the various complexes was investigated.

Diffusion studies with thin collagen membranes indicate that the rate of diffusion in non-agitated systems is very slow. The diffusion constant is dependent on thickness, temperature and basicity and independent of the chrome concentration. Exchange studies indicate that the sulfate ion can be exchanged from the chromium complexes.

In other PL-480 sponsored research at Central Leather Research Institute, a new approach to vegetable tanning was investigated. The tanning sequence is reversed, that is, tanning commences in the hydrolysable tan and is completed in the condensed tan liquor. This method allows a saving of the costlier condensed tannins without detriment to the overall physical and chemical properties of the leathers.

Also at Central Leather Research Institute, studies under a PL-480 grant on the graft polymerization of vinyl monomers (methyl methacrylate, acrylonitrile, and methyl acrylate) onto soluble collagen and hide powder have continued. Evidence that the products are true grafts was provided by turbidometric titration, swelling behavior in various solvents, intrinsic viscosity in mixed solvents, and infrared spectra of copolymer and its hydrolyzates. Modified collagens were used to gain evidence for the grafting sites in this process, but the results did not provide unequivocal proof of the functional groups involved in the grafting. The hydroxy amino acid residues and the peptide backbone of the protein are indicated as sites for initiation of grafting. Dinitrophenylated, acetylated, methylated, deaminated, cyanoethylated, N-brominated, vinylated, thiolated, and dialdehyde starch tanned modified collagens were studied. The methods for grafting used for ground up or soluble collagen were not suitable for intact hide. Use of emulsion polymerization techniques with ceric nitrate gave satisfactory results with methyl methacrylate, methyl acrylate, and acrylonitrile. A mixture of anionic and nonionic surfactants gave better results than either alone; cationics alone or in combination were not effective. The grafted pelts showed an increase in shrinkage temperature and had a "tanned" appearance. Continuing studies at EU on new tannages and mechanisms showed that tris (hydroxymethyl) nitromethane, unlike pentaerythritol, exhibits strong electron-withdrawal by the nitro groups, which enables it to

react with resorcinol and tan skins in dilute aqueous solution at room temperature.

The Mannich reaction, using formaldehyde and malonic acid, was studied further using model compounds and proteins other than collagen. The  $\epsilon$ -amino group of lysine residues was involved in this interaction with proteins and several of the derivatives were identified. One of these contains two lysine residues demonstrating the presence of a covalent cross link. Studies on the interaction of glutaraldehyde with collagen have continued. Chromatography as a tool to isolate the unknown derivatives from the protein hydrolyzate has failed because of the labile nature of these unknown products.

Hydrothermal shrinkage of collagen and leather is being studied under a PL-480 grant at Central Leather Research Institute, Madras. Hide protein is denatured by moist heat which is manifested by shrinkage of this fibrous protein. Little is known about the dimensional changes that occur in this phenomenon. Limed skins and pickled skins at pH extremes of 12 and 2 respectively shrink less when heated in water than raw or delimed skins which are at an intermediate pH range. This study indicated that diffusion, deposition of materials, stabilization of structure as by tanning, and drying influence the shrinkage temperature ( $T_s$ ) and the area of shrinkage. Diffusion may lead to swelling which results in a lower  $T_s$  and less area shrinkage. Effects of swelling can be avoided by using process liquor instead of water when determining  $T_s$ . Tanning introduces crosslinks that stabilize the structure of the fibrous protein and thus raises the  $T_s$ . Area shrinkage of vegetable tanned hide is higher than that of chrome, oil, or formaldehyde tanned skins. Determination of the number of cross links introduced by tanning indicated that these have an effect on the heat of fusion value of the protein.

In PL-480 sponsored research at British Leather Manufacturing Association, Milton Park, investigations on the effects of dry heat on collagen suggest that the degradation is similar to that induced by  $\gamma$ -irradiation. The main mechanism of breakdown is oxidative in character and leads to loss of amino acids and chain scission. Tanning agents appear to have little effect on rate of breakdown but mitigate the effect of protein breakdown.

In supporting research under a contract at Northwestern University, Evanston, Illinois, the interaction of anhydrous formic acid with model amides showed that multiple equilibria are present. Although hydrogen bonding was universally prevalent, protonation of amides occurred only at low acid concentrations. These reactions involved the acidic proton of formic acid and amide carbonyl oxygens. The extent of protonation was proportional to the basicities of the amide carbonyls involved. The information thus obtained was applied

to the solubilization of collagen and its disassembly into components which formed high molecular weight polymers, notably  $\gamma$ -gelatins. Collagen-fold formation was studied by comparing the kinetics and development of fibers from different polymeric gelatins via the decay of absorption of the amide II bond. It was found that water binding ability followed fold formation.

In research at EU a device has been constructed to obtain infrared spectra of films of native collagen under conditions of precisely-controlled humidity and temperature. The study of the interactions of amino acids in aqueous solution by NMR continues. The aliphatic vicinal coupling constants for tyrosine, tryptophan, and histidine in basic aqueous solution have been obtained as a function of temperature and concentration. The results have been interpreted in terms of the variations in relative energy of the classical staggered rotamers. The study of the above amino acids in different solvent systems are in progress.

Further studies at EU with the silver sulfide plated silver billet electrode proved that this electrode responds only to the sulfide ion and is not affected by the hydrosulfide ion. Therefore, the electrode can be used to measure the un-hairing potential of a lime-sulfide un-hairing solution. An apparent pH dependence was traced to the hydro-sulfide ionization equilibria and an oxygen effect was found which necessitates rigidly controlled conditions or an inert atmosphere for accurate work. An electrodialysis cell for solid hide pieces was designed, constructed and evaluated for determining the total electrolyte content of a hide piece. Total cations and anions can be determined separately and the solutions can be analyzed for the separate cations and anions involved. Recoveries of 98-100 percent were obtained.

Elimination of Hide and Skin Defects. Sheep ticks (keds) were previously shown to cause the sheepskin defect known as "cockle". A test on 110 insect-free lambs, half of which were infested with parasitic keds, indicates the total economic losses to meat, wool, skins and leather will provide incentive for ked eradication. Carcass values were reduced by 9% and wool growth by 20%. The induced cockle defect reduced the value of pickled skins by 29% and of garment suede leather by 47%.

The seedy and ticky defects of sheepskin, seriously affecting some leathers, were both found to originate from the sharp awns of certain western forage grasses.

In cooperation with Veterinary Sciences Division, a survey of tannery hides in process revealed a high incidence of Demodectic mange in beef cattle, refuting its reported limitation to dairy breeds.

In cooperation with Animal Sciences Division, a study on freeze branding, soon to be completed, should result in improved procedures for minimizing hide damage while increasing brand legibility as an alternative to hot branding.

Histological study of hide biopsies from 60 twin heifers in a comparative feeding test will contribute to the interpretation of both genetic and nutritional effects on hide structure, with emphasis on vertical fiber defect. A penetrometer device for nondestructive detection of the vertical fiber defect in raw hides has shown good correlation with leather strength. If confirmed, this would have considerable practical value for presorting hides.

#### NEW AND IMPROVED MEAT, MILK, EGGS AND OTHER ANIMAL FOOD PRODUCTS

Collagen Dispersions for Food Uses. Considerable technical data necessary for estimating the cost of producing high solids collagen dispersions by comminution of cattlehides were obtained. Much effort was also expended on the development of a continuous operation. Techniques for preventing heat denaturation of the collagen during comminution were tested. A computer program for calculating total and balanced essential amino acid content of mixtures including collagen in any proportion has been worked out.

Previous work had shown that comminuted collagen could be mechanically dispersed after mild acid swelling (pH of about 4.0). This necessitated subsequent neutralization for stability and edibility. Alternatively, two procedures have been found for dispersing neutral suspensions of collagen without losing its fibrous properties. Both of these use mechanical action; one is carried out with heat while the other procedure is conducted at room temperature after first mixing with guar gum.

Collagen was found to be a useful binder and texturizer for other proteins. Collagen-soya flour mixtures are undergoing nutritional evaluation. Glyceraldehyde, a naturally occurring non-toxic aldehyde, fixes collagen and imparts heat stability to films. Such films are only two-thirds digested.

In supporting research, electric birefringence experiments on solubilized calfskin collagen show that collagen undergoes a specific type of polymerization in an electric field. This might be extended to form the basis of a novel technique for the synthesis of polymeric films and fibers.

Other supporting research is conducted under a PL-480 grant at University of Turku, Turku, Finland. The studies show that

the mammalian carcass contains various types of collagens which have their counterparts in the lower animals. This concept increases the understanding of the relationship between the structure and function (tensile, supporting, filter) of collagens. The intramolecular differentiation of the various chains in collagen during the evolution has been established. It was also demonstrated that "insoluble collagen" contains accessory proteins which may have a role in the organization of tropocollagens to insoluble structures. It was shown that collagen of a living animal can be influenced both quantitatively and qualitatively by such factors as a variation in the oxygen supply, X-ray irradiation, various common drugs and growth hormone. The production of collagen is even more sensitive to adverse agents than the formation of other proteins in the body.

PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISM AND  
NATURALLY OCCURRING TOXINS

Protecting Animal Feed Supplies. A study is continuing on the comparison of several isolation techniques for salmonellae in meat and bone meal. The micro-method developed for identifying single colonies of Enterobacteriaceae with an incubation time of one day was employed in comparing sampling efficiency. Results indicate that assay of a single large sample of meat and bone meal may provide control comparable to that achieved with numerous small samples.

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## POTATO UTILIZATION

### Problems and Objectives

An increasing proportion of U. S. potato production is being processed into products such as chips, French fries and dehydrated potatoes. The welfare of the potato industry depends on continuing this trend. Processing of potatoes, however, poses several problems, the most important being disposal of potato wastes. The potato starch plants, which provide growers and processors with an outlet for cull potatoes, as well as other processors may be forced out of business by antipollution laws. Recovery of useful materials from potato wastes would help alleviate the potato waste problem and provide additional return to the processor. Predicting and controlling processing characteristics of potatoes is another area of concern. Potatoes placed in cold storage to extend the processing season require a lengthy reconditioning treatment which is not always successful. Research is also needed to provide new and improved processed potato products.

Major objectives of present research are:

1. Developing technology for recovery of useful materials, including proteins and amino acids, from potato waste.
2. Developing technology for evaluating potatoes for processing, controlling browning during processing, and predicting quality at time of consumer use.
3. Perfecting the explosion-puffing process to provide new and improved dehydrated potato products.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

Sugar Enzymes. Sucrose synthetase activity per tuber increased sharply during the period of cell division, leveled during cell enlargement, and declined sharply after maximum tuber size was reached. The physiological role of sucrose synthetase is not clear, but it may be a maturity index. "Immature" tubers do not recondition as well as "mature".

Sucrose phosphorylase activity was found in Kennebec and Norchip tubers and followed through the growing season. It is higher in reconditioned tubers after cold storage. The possible use of callus cultures to elucidate enzymic interrelationships is being examined. At the College of Agriculture, Krakow, Poland, under a PL-480 grant, an investigation is underway using standard Polish and American varieties of potatoes to determine sugar accumulation in cold storage

and sugar transformations in warm storage. Preliminary results indicate that there was no significant difference between the Polish and American varieties used in the investigation.

Potato Proteins. Potato proteins are greatly similar and inherently difficult to separate on a preparative scale. No pure single protein was obtained. Disc electrophoresis, revealing 20-30 components, was used to show that protein patterns of a variety were constant through variation of agronomic factors; however, minor changes due to tuber storage somewhat reduces the value of disc electrophoretic patterns for variety identification. Analytical support was given to a Crops Research breeding project. The breeding of potatoes for significantly higher protein content was shown to be a possibility.

The reactions of 16 potato amino acids with glucose were studied using a specially developed procedure of frying paper "chips" followed by ion exchange chromatography. The study included numbers and types of intermediates formed, rate of reaction and loss of nitrogen, and decomposition of the intermediates. Gamma aminobutyric acid is the most reactive and probably most important in relation to potato chip browning, since it is third in abundance, while asparagine and glutamine, more abundant, react more slowly. Fructose reacts as rapidly as glucose, but less amino acid is lost; the higher glucose content in tubers makes it more important. Pentoses, whose occurrence is less well known, react faster than either fructose or glucose. Minor amounts of aminodeoxy sugars accumulate during the frying of light chips. High-sugar slices give larger amounts of aminodeoxy sugars which appear early and then disappear as brown polymeric colorant appears, giving a darker chip. High-sugar chips finished by microwave processing, in which heat exposure is reduced during the last stages of frying, show larger amounts of colorless intermediate, with little polymer formation.

Processing Quality. The durometer, which measures the penetration of a pointed plunger, is being studied as a possible field instrument to evaluate the suitability of potato raw stock for French fry manufacture and to provide advance warning of a susceptibility to deterioration in storage that leads to production of French fried potatoes of low textural quality. Such deterioration does not occur with all lots of varieties.

In a search for a potato variety chipping directly from 40°F storage, 1500 seedling plants from 22 crosses were harvested and tested, and 93 produced acceptable chips from 40°F storage. Their parentage have been identified and out-crossed with tuberosum cultivars, producing 3500 seedlings which will be evaluated. Field plants inoculated and infected with virus X were not found to have better chipability.

Explosive Puffing. Pilot plant studies are underway to discover the effect on product flavor of puffing temperature and of raw material reducing sugars. During explosion-puffing research in model systems, off-flavor intensity was found to be dependent on reducing sugar content, moisture content and pH, but not on SO<sub>2</sub> content. Off-flavor decreased with increasing moisture content and with decreasing pH. The use of nitrogen gas in the superheating steam reduces "off-flavors" to tolerable levels. Ascorbic acid was found more effective than other acids in reducing "off-flavors". The "off-flavor" was attributed to Strecker degradation of aldehydes, alkyl-pyrazines and products of sugar pyrolysis.

Two storage tests are in progress to determine whether the "off-flavor" resulting from puffing of potato dice increases during storage. One lot was made from high sugar content potatoes; the other from low sugar content potatoes.

#### ALLEVIATION OF SOIL, WATER, AND AIR POLLUTION AND DISPOSAL OF WASTES

Potato Starch Factory Wastes. A survey of starch factories, made to determine the composition of protein water effluent showed a solids content ranging from 1.5-2.8%. Protein water prepared to have the same composition as that found in the survey was used to study sedimentation of proteins coagulated by steam-injection heating. The effect of soluble solids and pH on percent protein coagulated, settling mechanism, retention time and protein purity were evaluated. Slurries were readily filterable in a plate and frame press at pH 3.0-5.0 and temperatures of 50-180°F. Synthetic polyelectrolytes were found to be ineffective in precipitating proteins. It was shown that a residual protein level of 180 ppm or less was required for the efficient operation of the ion exchange columns used to recover other valuables. This work has shown the possibility of quantitatively recovering the proteins by steam injection heating to 180-220°F at a pH of 3.0-4.0, followed by conventional solid-liquid separation methods, after which the other constituents may be removed by ion exchange.

Coarse membrane reverse osmosis modules have been tested for protein removal from the waste stream, but flow rates were considerably lower and small molecule retention was higher than expected. A batchwise rather than a continuous mode is more efficient for reverse osmosis pre-concentration of the protein water for subsequent processing. Ion exchange removal of cations before recovery of the amino acids improves the recovery of both materials. Process variables (temperature, flow rates, volumes, concentrations, regeneration levels) are being optimized for ion exchange recovery of fertilizer salts, amino acids, and organic acids and processing control instruments are being developed.

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## VEGETABLE UTILIZATION

### Problems and Objectives

The trend toward processing greater amounts of the vegetable production continues to accelerate under the impact of economic and social factors. Economically, utilization as processed, rather than fresh, vegetables provides a constant source of supply with less price fluctuation. Modern military feeding needs products with high bulk density which do not require refrigeration and are rapidly hydratable. Consumer preference is shifting to "convenience" foods. Therefore, the emphasis in research is on improvement in processing technology, particularly for dehydrated products.

The major objective of this research is to perfect the explosion-puffing process to provide dehydrated vegetables which rehydrate rapidly, cook in the same or less time than their fresh counterpart, and have good initial flavor and storage stability at room temperature.

### Progress - USDA and Cooperative Program

#### NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

Dehydrated Products. Explosion puffing studies (batch) on processing varieties of onions, in cooperation with Gilroy Foods Company, California, yielded promising products with rapid rehydration rates in 180°F water. This research is continuing, cooperatively, with Gilroy supplying varieties otherwise unavailable in produce markets. Optimum processing conditions are being determined and testing of the product is underway. Red bell peppers of thick-fleshed processing varieties were successfully batch puffed in pilot plant studies. Pepper pieces, partially dried to 12-19% moisture, were puffed at 30 psi gun pressure, and after rapid final drying, rehydrated quickly to a product of excellent quality. This research was carried out in cooperation with Gentry Foods Corporation, California. Gentry will supply other vegetables of processing varieties, now air-dried commercially, in which they and other firms are interested in obtaining a product which rehydrates quickly.

Several hybrid carrot varieties, supplied by the Department of Horticulture, University of Wisconsin, were explosion-puffed and dried. Of the varieties tested, the hybrid "Spartan Bonus", in which the xylem (core) is nearly the same red-orange color as the phloem (outer tissue), yielded an excellent product. Red Core Chantenay and Imperator, the major varieties now processed, have yellow or green xylem tissue, objectionable in the final product.

Utilizing the process developed at EU for hot-air drying of mushroom pieces, the Kennett Canning Company of Kennet Square, Pennsylvania is now producing dry cultivated mushrooms commercially.

Publications - USDA and Cooperative Programs

NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

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## DECIDUOUS FRUIT AND TREE NUT UTILIZATION

### Problems and Objectives

It is essential that there be continued improvement in the quality of processed fruits if this commodity is to hold its own as an element in the national diet. Mechanical harvesting and other changes in the growing and harvesting of fruits create problems which must be met by improved processing methods. The development of new fruit varieties more suitable for various types of processing is an important factor in preserving markets for the fruit processing industry and protecting fruit growers against variations in price due to irregular yields from year to year.

Objectives of the research are:

1. To develop high quality dehydrated fruit products which may be reconstituted quickly, and which may be consumed as snacks.
2. To develop improved methods for the processing of mechanically harvested fruits and for maintaining the processing quality of fruits harvested by this method.
3. To evaluate the processing characteristics of new fruit varieties.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

Cherries. Volume buying of tart red cherries in water eliminated rehandling and damage due to weighing and led to the production of a 99% Grade A, scaldfree product. About 50 million pounds (20% of crop) were bought by volume in 1969 with an estimated saving of \$285,000, realized from a 3% increase in pack-out and improved quality of the product.

The use of an abscission-promoting chemical (2 chloroethyl-phosphonic acid) before mechanical harvesting has been found to improve recovery of sweet cherries for brining (maraschino cherries) from 60% to over 95%, making such harvesting practical. No changes were required in the commercial process for maraschino cherries from treated raw stock. Both processed yield and quality were fully equal to untreated lots. Immediate orchard brining maintains the quality of this fruit.

The identification of the components of commercial cherry essence has continued. A fraction which had a pleasant apple-like odor

yielded n-hexanol, 2-hexenol, 3-hexenol and 2-hexenal. Identification of terpenes in a subsequent fraction is in progress. Characterization of the typical Montmorency cherry flavor has begun using fruit rather than essence. Aroma develops after the fruit is crushed, indicating enzymic involvement. The "cherry preserve" flavor is largely non-volatile.

In research conducted under contract at Temple University, Philadelphia, Pennsylvania, the post-harvest metabolism of red tart cherries was studied in relation to the effect of bruising on the texture and quality of cherries for canning. Bruised and unbruised cherries were injected with 3 radioactive metabolites: C<sup>14</sup> labeled glucose, 2-C<sup>14</sup> acetate, and 1,5-C<sup>14</sup> citrate. Bruising caused an initial increase in total CO<sub>2</sub> output, followed by a decrease. However, bruising interfered with the normal metabolic cycles of the 3 incorporated metabolites. The fact that the metabolites were incorporated into the alcohol insoluble (polysaccharide) fraction of cherries indicates that alternate metabolic pathways were available. Bruising caused an increase in lignin and cellulose, but a decrease in the pectin fraction. The alcohol insoluble fraction showed a net increase. This finding is consistent with the observed increase in firmness and drained weight of bruised and aged red tart cherries when canned.

Apples. Cooperative tests were continued with Michigan State University to assay consumer reaction to explosion-puffed apple snacks. Panelists evaluated the apple snacks for taste and specific characteristics, i.e. flavor, color, piece size, texture and sweetness. The apple snacks were generally well received with 66% of the panelists making a favorable response. For all five characteristics, the panelists found the product to be acceptable. Cost estimates on the preparation of explosion-puffed apple pieces with a batch gun have been made and show that the process is competitive in product price with low-moisture commercial products.

The processing quality of apples salvaged from the orchard floor by a simple new USDA pick-up harvester has been found to be acceptable. The machine is now in commercial production.

Treatment of apples with hot isopropyl alcohol or, preferably, its vapors removes cuticle wax and allows a reduction in the time, temperature, and concentration of lye in subsequent lye peeling, greatly improving the process. Heat exposure, loss of tissue and the amount of caustic required are reduced while throughput quality of product, and yield are increased. Reduced tissue loss also reduces the waste disposal problem. The exposure time to the alcohol increases with increased apple cold storage time. There is a difference between varieties in the ease of peeling, with York most difficult and Golden Delicious easiest. The calyx must be removed

mechanically after peeling to avoid downgrading of sauce products due to calyx particles.

Pears.

In contract research at Rutgers University, New Brunswick, New Jersey, 37 new pear selections and 15 repeat selections, all from the 1968 harvest, were evaluated for processing quality (pH, acidity, solids, texture, color, flavor) before and after canning. Seven were highly acceptable, nearly equal to Bartlett, and were shown to the Mid Atlantic Food Processors Association. Several selections were evaluated for puree and three were found acceptable. From the 1969 crop, 45 new selections and 25 repeats were ripened and processed. These will be evaluated after the storage period.

Publications - USDA and Cooperative Programs

NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

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## TOBACCO UTILIZATION

### Problems and Objectives

Tobacco is grown on about a million acres, and in seven states typically provides more farm cash receipts than any other field crop. The most serious problem affecting the tobacco industry is the concern regarding effect on health.

Major shortcomings in developing a safer cigarette are the inadequacies of biological testing procedures and the failure to know which substances in tobacco and smoke should be removed. There is no practical procedure for determining which substances in tobacco and tobacco smoke are injurious to public health. Based on limited knowledge, some currently marketed cigarettes with charcoal and other filters apparently show selective reductions in certain properties in laboratory animals but cannot be considered a final solution. In addition, such cigarettes are deficient in flavor and aroma and meet with some consumer resistance. This problem requires a concerted effort to learn specifically what must be removed from cigarette smoke and how this can be done effectively without loss of desirable organoleptic properties.

Objectives of research are to:

1. Develop methods for determining which substances in tobacco and tobacco smoke are undesirable from standpoint of public safety, and
2. Develop technology for elimination of substances determined to be undesirable.

### Progress - USDA and Cooperative Program

#### REDUCTION OF HAZARDS TO HEALTH AND SAFETY

Fractionation and Bioassay of Cigarette Smoke Condensate and Tobacco Leaf Samples. Further fractionation of smoke condensate by countercurrent distribution of the neutrals between nitromethane and cyclohexane was continued. In all, ten kilos of cigarette smoke condensate will have been fractionated. At the same time, the weak acid fraction was subfractionated by steam distillation for biological tests. Fractions of unburned tobacco extracts have also been submitted for tests.

Assays, over a period of 50 weeks by the mouse-backpainting method, are conducted under contract by the Roswell Park Memorial Institute, Orchard Park, N. Y. This method, due to replications and length of

test period, requires preparation of large quantities of test material at EU. The third phase of the bioassay of smoke fractions is underway but it is still too early to report results. Four scientific papers have resulted from the first and second phases of the smoke work.

Tumor-promoting activity in a highly polar, methanol-soluble neutral fraction has been reported in one of these papers. With extracts of unburned tobacco, weak tumor-promoting activity is suggested by phase 2 experiments in two subfractions of the least polar crude fraction. The activity of hexane extracts in phase 1 was found to be due to the solvent mixture used in their application, and a scientific paper reporting this unexpected finding of activity in aliphatic hydrocarbons was presented before the American Association for Cancer Research in April, 1970.

Bioassays by mouse back painting were also continued at Lexington, Ky. in the Department's cooperative program with the University of Kentucky. Acute toxicity tests on aged condensates from the University of Kentucky Reference Cigarette indicate that animals will tolerate dosages as high as 450 mg/wk of whole and 500 mg/wk of acid extracted materials. The tumorigenicity of tobacco smoke condensates (TSC) was considerably lower than that of the authentic carcinogen, benzo(a)pyrene. This indicated that high doses over long periods of time are needed to assess the relative tumorigenicity of TSC and that it is important to select adequate population levels and suitable environments for the maintenance of a high surviving population during the latter phases of an experiment. There was little difference in the tumorigenicity of TSC derived from high or low nitrate burley cigarettes, but the latter evidenced a shorter latent period. The TSC derived from all burley cigarettes was less tumorigenic than that derived from a commercially blended cigarette. There appeared to be little difference in the tumorigenicity of acid extracted and whole condensates. When alkaloid toxicity does not allow the application of adequate dosage or the maintenance of suitable populations, acid extraction of TSC appears to be an acceptable measure to allow an experiment to be performed with the mouseskin procedure. An experiment to evaluate changes in variety and curing practice on the tumorigenicity of condensates from air-cured tobacco is continuing satisfactorily.

Deleterious Components in Cigarette Smoke Condensate. Major tumor-promoting activity in animal tests has been found in the neutral fraction of cigarette smoke condensate. Investigations were continued on the chemical identity of compounds, or groups of compounds, in this fraction in an effort to correlate biological activity with specific components. The eleven new constituents characterized during this report period included 4 alkylphenols and 7 alkylbenzonitriles. Although one of these compounds, 2-butylphenol, is known to have tumor-promoting activity it appears that the quantities present would account for only a minor portion of the overall fraction activity. Biological activity was also found in a more polar subfractions of the neutral fraction.

Most of the compounds in this latter subfraction had molecular weights in excess of 450. A 40% reduction in the acetic acid (a ciliostat) content of cigarette smoke and increased filter (charcoal) effectiveness for acetic acid and phenols was achieved by reducing the pH of cigarette smoke from 5.6 to 4.2. Increasing the smoke pH resulted in 80-90% reductions in  $H_2S$  and HCN in the vapor phase. A detailed study of the origin of the high molecular weight acidic pigments of smoke condensate yielded no strong evidence that the pigments are artifacts produced by smoke collection or experimental manipulation.

At the University of Kentucky Research Foundation, Lexington, several composition studies are conducted under contract. In a study of higher-boiling heterocyclic nitrogen compounds, further evidence was obtained for the presence of two new compounds in tobacco smoke, i.e.  $\gamma, \gamma'$ -dipyridyl and 2,2'-dipyridylamine. The presence of nitrosamines in smoke condensate has not been demonstrated. Using known controls, research was continued on development of an improved analytical procedure. Twenty-one nitrosamines, of aliphatic and/or aromatic nature, were converted to the corresponding hydrazines by reduction with lithium aluminum hydride. The hydrazines were subsequently reacted with 9-anthraldehyde or phenanthrene-9-carboxaldehyde to form the corresponding hydrazones. All of the hydrazones exhibited pronounced fluorescence at 350-450  $\mu\text{m}$ . Application of this derivative procedure to a neutral fraction of tobacco smoke yielded a compound having chromatographic and spectral characteristics of N-nitrosopiperidine. A spray reagent test (diphenylamine plus palladium chloride) applied to neutral fractions of three different cigarette smoke condensates yielded a positive test in only one of the samples. Smoke condensates prepared by (1) collection on a Cambridge filter, and (2) condensation in cold glass traps, were subjected to trace element analysis. More bromine, cesium, scandium, zinc, iron and cobalt were found in (1) while more lanthanum, chromium, mercury, selenium, silver and antimony were found in (2). Arsenic levels were below detection limits. Tobacco and paper of the Kentucky Reference Cigarette were analyzed for 12 trace elements. From the analysis of smoke condensate and ashes prepared from filter and non-filter cigarettes, it was seen that for all the elements studies, the filter cigarette is much more effective in preventing the transference of these trace elements from cigarettes into smoke than the nonfilter cigarette of the same brand.

Pyrolytic Formation of Deleterious Components. In research at EU, determination of precursors for various smoke constituents was continued as follows: 1) Extracts, obtained by the sequential extraction of tobacco with solvents of increasing polarity, were pyrolyzed. Aromatic hydrocarbons were generated primarily by the hexane and acetone extracts; phenols by the ethanol extract and final leaf residue; and nicotine degradation products by the ethanol extract. Products generated were related to the chemical composition of the extracts pyrolyzed.

2) Sodium acetate, on pyrolysis, was shown to produce relatively large quantities of 3,5-xlenol, a constituent of smoke. 3) Compounds, related structurally to caffeic acid and other cinnamic acid derivatives found naturally, were pyrolyzed at temperatures ranging from 500-900°C. More than 20 aromatic hydrocarbons, predominantly polynuclear aromatic hydrocarbons, were generated. The composition of the product mixtures was influenced, both qualitatively and quantitatively, by the temperature of pyrolysis. Results could be explained by a multiplicity of mechanistic pathways involving nonfree radical, as well as free radical, intermediates.

Related studies were conducted in the contract research at the University of Kentucky. Amino acids were pyrolyzed at 650° and 850°C. Gases formed during pyrolysis, including hydrogen cyanide and carbon monoxide, as well as carbon dioxide, methane, ethane, ethylene and ammonia, were identified and their amounts determined. Gases from sulfur containing amino acids, such as methionine and cystine, also contained carbon disulfide and carbon oxysulfide. Variations in the structures of amino acids affect the chemical composition of the pyrolyzates. Thus, an increase in chain length resulted in an increase in carbon monoxide and polynuclear hydrocarbons. The presence of additional functional groups resulted in an increase in hydrogen cyanide, heterocyclics and polar compounds (largely nitriles and phenols). Proline, an atypical amino acid, gave relatively large amounts of hydrogen cyanide and heterocyclics. A preliminary determination of benzo[a]pyrene in the pyrolyzates of valine indicated that this compound is formed at both temperatures. The herbicide, maleic acid hydrazide, was pyrolyzed at 850° in a nitrogen atmosphere. Carbon monoxide, hydrogen cyanide, and ammonia were found among the gases of pyrolysis. The condensable pyrolysate consisted of aromatic nitriles, nitrogenous bases, phenols, and aromatic hydrocarbons including benzopyrene. The formation of carbon monoxide, hydrogen cyanide, ammonia, phenols, and benzopyrene from maleic acid hydrazide has a direct bearing on the problem of tobacco and health because of the extensive use of the herbicide for the prevention of sucker production in tobacco plants.

Modification of Smoke Composition. Approximately 27,000 sample cigarettes were made from Reference Cigarette Tobacco treated in the cigarette manufacturing laboratory at the University of Kentucky with ammonium meta-vanadate or potassium chlorate. (In a recently discontinued cooperative agreement research project, it was established that ammonium meta-vanadate-treated cigarette tobaccos yielded increased amounts of phenol and benzo[a]pyrene in the smoke; potassium chlorate-treated tobaccos yielded decreased amounts of nicotine, phenol, and benzo[a]pyrene). More than 137,000 cigarettes, commercial and sample types were smoked, which yielded nearly 6000 grams of condensate. Eight Mason MK 111 smoking machines are operational, giving the laboratory a maximum daily smoking capacity of about 5500 cigarettes. A new trapping and condensate recovery system (based on the Elmenhorst

system) was initiated and is in use together with the system used previously. The new system allows more rapid recovery and more readily-soluble condensate. Smoke data from thirty chemical treatments of tobacco were compared to the constituent levels obtained from the smoke from untreated cigarettes. The quantities of phenol varied from 70% to 20%; o-cresol, 59% to 335%; m-,p-cresol, 57% to 273%; and benzo[a]pyrene, 48% to 191%.

Correlation coefficients relating the levels of various leaf and smoke components to thermal analytical data were determined. A direct correlation was found between both the level of nicotine delivery per cigarette and the weight loss of tobacco below 350°C. There was an inverse relationship between the level of nitrate in the leaf and both the low and high temperature weight losses.

Modification of composition of cigarette smoke by changes in processing steps, such as aging and fermentation, is also being investigated. Chromatographic methods were developed for the isolation of biologically important compounds from cured and fermented tobacco, including thin layer, ion exchange, gel filtration and adsorption. Colored and fluorescent interfering materials are removed by adsorption on polyvinyl pyrrolidone. Investigation was made of enzymatic activity in processed tobacco with major emphasis on peroxidase, which persisted in aged samples. Peroxidase decreases 40-60% during curing. The activity is originally divided between two isozymic bands, one of which disappears on aging. Peroxidase activity can be used as a sensitive test for enzyme protein. Electron microscopic examination of leaf structure showed drastic changes due to processing from green through fermented tobacco. Preliminary studies indicated the absence of any known mycotoxins in mold infected tobacco.

Development of New Bioassay Methods. In contract research at the University of Kentucky Research Foundation, Lexington, studies continue on biological systems and on their response to tobacco constituents in evaluating their use as a bioassay procedure to replace or augment the mouse-back-painting method. The following systems have provided basic information of potential value in the development of new and improved bio-assay procedures:

- (1) Rodent tracheal rings can be maintained in a viable state in suitable culture media. The cilia of these rings have shown a dosage response to nicotine and cigarette smoke condensate.
- (2) Multiple intravenous injections of dimethyl benzanthracene in rodents resulted in discernible breaks in the chromosomes of cells in the marrow, liver, and spleen. This rapid biological response could provide the basis for bioassay of cigarette smoke carcinogens.

- (3) Ingestion of an aqueous extract of cigarette smoke condensate appeared to sensitize rodents to subcutaneous injections of the carcinogen benzo[a]pyrene. Preliminary results indicated the LD<sub>50</sub> benzo[a]pyrene dose was within the range of 1-6 micrograms.
- (4) The membrane-bound electron transport system of Staphylococcus aureus was found to be sensitive to cigarette smoke constituents. Benzo[a]pyrene, a carcinogen, inhibited the electron transport system while benzo[e]pyrene, a noncarcinogen, had no effect.
- (5) Pith cells from the stalk of N. tabacum proliferate when  $10^{-8}$  to  $10^{-6}$  molar concentrations of benzo[a]pyrene were added to the culture medium. Benzo[e]pyrene was ineffective in these tests.

Collaborative Analytical Studies. A comprehensive collaborative study of the method for determining tar and nicotine in cigarette smoke was conducted during the past year. All leading tobacco companies participated and each performed 120 measurements of five characteristics. The data were analyzed by Biometrical Services Staff, ARS, to determine the effect of different variables on the results obtained. Significant variables were "within" and "among" laboratory differences and laboratory X material interaction. The method proved to be satisfactory for all types of cigarettes except those with high tar and nicotine delivery. A study is now under way to try to correct this deficiency.

#### NEW AND IMPROVED FEED, TEXTILE, AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Increased Flavor and Aroma in Cigars. Work has continued on the isolation and identification of constituents responsible for the odor of cigar butts. The vacuum distillate from cigar butts possesses the typical butt odor and is highly complex in composition when examined by gas chromatography. A strong similarity is seen between the composition of the butt distillate and the distillate from cigar smoke total particulate matter. On separation of the butt distillate components by solvent extraction, all subfractions have odor but more of the undesirable odor is found in the bases, which contain a substantial portion of simple pyridine derivatives. Evaluation of results to date has been handicapped by the poor resolution obtained with conventional large bore gas chromatographic columns. It is planned to employ open tubular columns in future work. This study is being performed under cooperative agreement with the Cigar Manufacturers Association of America (CMA).

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## MAPLE SAP AND SIRUP UTILIZATION

### Problems and Objectives

Maple sirup producers are largely full-time small farmers, in an area encompassing 14 states from Maine to Minnesota and south to Virginia. These producers are utilizing less than 3% of the available sugar maple trees for sap production. Extensive stands of untapped trees are located in agriculturally depressed areas and since, under proper conditions, maple sirup can be a six-week seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products, these trees represent a good potential source of cash income in these areas. The production of maple specialty items is another potential source of cash income for the farmer or producer. Sustained effort is required to obtain the information needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner.

Major objectives of the research are:

1. Improving technology and reducing cost of sap collection and processing.
2. Improving quality of maple sirup and other maple products.
3. Developing new maple products.

### Progress - USDA and Cooperative Programs

#### NEW AND IMPROVED FOREST PRODUCTS

Resazurin Dye Test. The resazurin dye test, which is used to quickly estimate microbial contamination levels in raw milk, has been modified to meet the needs of central maple sap evaporation plants. It was found that sap is a relatively poor culture medium and consequently the metabolic activity of the contaminating organism is too low to produce the rapid blue to pink color change required for a positive test reaction. Addition of nutrient broth to the sap and a one-half hour incubation of the mixture before adding the dye solution resulted in a test which indicates by color change within one hour the presence of  $10^7$  organisms per milliliter of sap.

Pasteurization by Ultra-violet Irradiation. Prevention of bacterial growth in maple sap is of importance in increasing sap farming and in making the central evaporator concept more attractive. Previous studies of static storage of maple sap have shown that direct ultra-

violet (UV) irradiation of the sap could be effective in reducing bacterial growth. To further these studies, an inexpensive trough-type UV sap irradiation unit was constructed and tested. It was found that effective pasteurization of maple sap could be obtained in a 9-foot, 3-light trough with sap flowing in a 1/2-inch depth at 480 gallons per hour.

Reverse Osmosis. Field tests of the reverse osmosis equipment in the 1969 season, using both new and old modules, resulted in dropping flux rates and in slime accumulation on the feed side. This indicated a need for better in-plant cleaning. The slower-flowing older modules had greater sliming than the newer type modules. After-season cleaning and storage restored the flux rate.

The reverse osmosis unit was field tested for the third (1970) season using membranes supplied by a cooperator to balance the equipment. Over 100,000 gallons of sap were processed with 50% water removal at 450 psi. Better flow rate and improved in-place cleaning reduced the slime accumulation and maintained flux rates. The original membranes, no longer commercially available, began to fail in the latter part of their third season's use.

It was found that increasing the temperature of the sap from 45° to 75°F can increase flow rates by 50% in reverse osmosis processing.

Reverse osmosis was found to be a convenient and practical laboratory method for pre-concentration of the sap without exposure to heat. The sap was concentrated to 27% solids preliminary to studies of flavor precursors.

Analytical Methods for Formaldehyde. The analysis for formaldehyde in maple sirup is being studied to determine whether there is possible interference in the analysis by flavor components in the sirup. This study was brought about by apparent excessive formaldehyde levels in New York maple sirup, thought to arise from pellets used to sanitize tapholes. Three components of maple flavor, methylglyoxal, glyoxal and acetol, were found to interfere with the analysis to some extent. Examination by GLC of cleaned-up fractions from the analytical method indicates that two reacting compounds are present.

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